Sara Tomás Sequeira

Bachelor in Chemical and Biochemical Engineering

Comparative study of particle size distribution analysis by laser diffraction between dry and wet dispersion methods and scanning electron microscopy

Dissertation submitted in fulfilment of requirements for degree of Master in Chemical and Biochemical Engineering

Advisor: Doutor Sérgio Silva, Scientist – Particle Engineering, Hovione Farmaciencia, SA

Co-advisor: Prof. Doutora Ana Isabel Aguiar Ricardo, Professora, Faculdade de Ciências e Tecnologias – Universidade Nova de Lisboa

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"I cannot fix on the hour, or the spot, or the look or the words, which laid the foundation. It is too long ago. I was in the middle before I knew that I had begun."

Jane Austen

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RESUMO

Um fármaco pode fisicamente apresentar-se em diferentes formas farmacêuticas como por exemplo pós secos, dispersões líquidas ou dispersões semi-sólidas. O tamanho das partículas que o constituem é considerado um parâmetro de extrema importância, uma vez que afeta um rol de características do API como a reatividade, a estabilidade, a eficácia, a textura, a escoabilidade, a viscosidade e a densidade, etc.

Formulações de APIs administradas, por exemplo, pela via respiratória ou oftálmica são normalmente compostas por partículas pequenas com tamanho inferior a dez micrómetros. É graças à utilização de técnicas de engenharia de partículas que é possível atingir a gama de tamanhos desejada. Ao longo desta tese foram estudados e caracterizados diversos produtos (API's e um excipiente farmacêutico), antes e depois de processados por técnicas de engenharia de partículas.

De modo a avaliar a distribuição do tamanho das partículas dos produtos, foram escolhidas duas técnicas de difração laser e uma técnica de microscopia eletrónica.

As técnicas de difração laser usadas partem dos mesmos princípios teóricos, no entanto diferem no algoritmo matemático usado para a geração de resultados na forma de uma distribuição de tamanho de partículas.

Para o método de dispersão líquida, os equipamentos utilizados foram um Malvern Mastersizer Hydro 2000 S e um Malvern Mastersizer 3000 Hydro MV. Para a análise por meio de dispersão seca o equipamento utilizado foi um Sympatec (constituído pelas unidades Helos, Rodos/M e Aspiros).

Um microscópio eletrónico de varrimento de marca e modelo Phenom ProX Generation 5 foi utilizado para se obter uma caracterização adicional em relação ao tamanho e morfologia das partículas.

Durante o decorrer deste trabalho comparam-se as três mencionadas técnicas sempre que possível.

Uma vez que as duas técnicas de difração referidas usam modelos matemáticos diferentes é esperado que os valores de PSD obtidos não sejam exatamente coincidentes. Deste modo todos os requerimentos do tamanho de partícula devem ter um método de análise associado validado.

Palavras-chave: API, engenharia de partículas, tamanho de partícula, difração a laser (dispersão líquida e seca), SEM.

ABSTRACT

An Active pharmaceutical Ingredient (API) may assume several different physical forms such as dry powders, wet dispersions and semi-solid dispersions. Depending on the route of administration, the size of API particles may be considered a critical attribute, since this property may affect a variety of product characteristics, such as reactivity, stability, efficacy, texture, fluidity, viscosity, density among others.

Fine API particles below ten micrometers in size, are required for pharmaceutical formulations administered by the pulmonary and ophthalmic routes. To achieve the desired particle size, several particle-engineering techniques may be employed.

In this thesis, a variety of products (API's & excipients) essentially intended for use in inhalation and ophthalmic formulations were characterized regarding particle size before and after being processed by particle engineering techniques.

In order to evaluate the particle size distribution of the studied products, two laser diffraction techniques and an electron microscopy technique were used.

The laser diffraction techniques used are based on the same theoretical principles however the mathematical algorithm used for data conversion and the particulate dispersion medium are different.

For analysis by wet dispersion, the equipment used was Malvern Mastersizer Hydro 2000 S and Mastersizer 3000 Hydro MV. For the analysis by dry dispersion, the equipment used was Sympatec (comprising the units Helos, Rodos / M and Aspiros).

Further characterization regarding particle size and morphology was carried out in a Phenom ProX Generation 5 microscope, a scanning electron microscope (SEM).

During the course of this work, whenever possible, the above mentioned techniques were used and a comparison between them was done.

Since these two laser diffraction techniques use different mathematical models for generating PSD data, it is expected that the PSD values reported are not exactly coincident. This means that every PS request should be always reported to a validated method.

Keywords: API, particle engineering, PSD, laser diffraction (wet and dry dispersion), SEM.

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GLOSSARY

Multi-scattering effect = laser light scattered by more than one particle before hitting the detector. These multiple scattering events cause the laser light to be scattered to higher angles. Higher angle scattering is associated with finer particles so multiple scattering causes a misguided estimation of the particle size [1].

Potent APIs = API or intermediate with an occupational exposure limit (OEL) at or below 10 ug/m^3, with high selectivity (i.e. ability to bind to specific receptors or inhibit specific enzymes) and/or the potential to cause cancer, mutations, developmental effects, or reproductive toxicity at low doses (at or near the therapeutic dose or lower) [2] .

ACRONYMS

API = Active Pharmaceutical Ingredient

cGMP = compliant with Good Manufacturing Practices

DPI= Dry Powder Inhaler

Malvern 2000 = Malvern Mastersizer HYDRO 2000S

Malvern 3000= Malvern Mastersizer 3000 Hydro MV

Mv = Malvern

FPP = Finished Pharmaceutical Product

OEL = Occupational Exposure Limit

PS = Particle Size

PSD = Particle Size Distribution

PSD parameters = d(0.1), d(0.5) and d(0.9)

RSD = Relative Standard Deviation

SD = Standard Deviation

SEM = Scanning Electron Microscope (Phenom ProX)

SRM = Starting Raw Material

Spt = Sympatec

Sympatec = HELOS + RODOS/M + ASPIROS

µm = micrometers

US = ultrasounds

WP = Wet Polishing

1. Introduction

1.1 APIs

An Active Pharmaceutical Ingredient can be defined as “ substance used in a finished pharmaceutical product (FPP), intended to furnish pharmacological activity or to otherwise have direct effect in the diagnosis, cure, mitigation, treatment or prevention of disease, or to have direct effect in restoring, correcting or modifying physiological functions in human beings" [3].

APIs assume three different forms in the majority of pharmaceuticals such as dry powders, liquid and semisolid dispersions. Their ranging size varies from nanocolloids to millimeter-size granules, depending on the dosage form and on the delivery pathway.

APIs in a crystalline form allow a variety of delivery routes such as oral, pulmonary, parenteral, and ophthalmic. Crystalline particles have a number of favorable properties that make their use in pharmaceutical drug formulation very attractive namely the intrinsic purity (due to the strict regularity of the crystal lattice) and the chemical stability against degradation (such as oxidation) [4].

An API in an amorphous state generally dissolves faster and has better compression properties. However, it presents as main problems lower physical and chemical stability than crystalline state, higher hygroscopicity then the last and variability from batch to batch [5].

The incorporation of an API into a drug product is a formulation development process. This encompasses an array of parameters. While biological activity is a prerequisite for a successful dosage form, it is not the sole determinant. Factors such as stability, processability, delivery, and availability to the target organ contribute to an efficacious pharmaceutical system. Optimization of these factors is a key development task, and the final product is often a compromise between pharmaceutical and practical (i.e., economic/engineering) considerations [6].

To be suitable for administration, drug products generally contain both API and excipients.

1.2 Excipients

According to the International Pharmaceutical Excipients Council (IPEC), "excipients are substances added to pharmaceutical formulations to help in processing, manufacturing and protection, give support, enhance stability, bioavailability and patient acceptability, assist in product identification and improve safety features or the effectiveness of the drug delivery system" [7].

Typically excipients are pharmacologically inert substances and the reasons why they are included in drug formulations are very different: dose compliance, improve solubility and control of API bioavailability, protection of the product from degradation and increase the robustness and reproducibility of production processes [8, 9].

For an excipient to be considered ideal it should ensemble specific characteristics such as being chemically stable, inert, non-toxic and inexpensive, having efficiency regarding its intended use and biocompatible [10].

Excipients are divided according to its various functional classifications depending on the role that they are intended to play, including, but not limited to [7]:

Table 1-1 Examples of Excipients functional classifications.

Excipients functions	
• Enhance absorption	• Compression filler
• Disintegration agent	• Sweetener
• Binding agent	• Stability
• Lubricant	• Antioxidant
• Compaction filler	• Preservative
• Filler diluents	• Buffers

In the case of dry powder inhaler (DPI) formulations, excipients act majority as API particle carriers. Generally, for an effective drug delivery by inhalation, no more than a few milligrams are needed, so excipients act as bulk providers improving the drug uptake and absorption.

Lactose is one of the most common excipients used in inhalation formulations. Lactose is highly crystalline, has smooth surfaces and satisfactory flow properties that makes it desirable to be used as particle carrier. It became the first and only excipient used in DPIs marketed in the United States [6].

1.3 The importance of particle size in pharmaceuticals

The particle size and shape can influence a great variety of critical physical characteristics, manufacturing processability and quality attributes. These properties ultimately affect the safety and efficacy of drugs.

Most drugs after crystallization may have to be comminuted and this physical transformation is required to various extents, often to enhance processability or solubility, especially for drugs with limited aqueous solubility.

The mechanisms by which milling enhances drug dissolution and solubility include alterations in the size, specific surface area and shape of the drug particles as well as milling induced amorphization and/or structural disordering of the drug crystal (mechanochemical activation).

Fine drug particulates are especially desired in formulations designed for parenteral, respiratory and ophthalmic use [12]. For a drug administration by inhalation, particle size is one of the most critical parameter to ensure an efficient therapeutic effect. There are three main aerosol deposition mechanism for pulmonary delivery of drugs such as inertial impaction (for particles with a size higher than five micrometers), sedimentation (for particles with a size between one and five micrometers) and Brownian diffusion (for particles with a size lower than one micrometer). Figure 1-1 shows the range of the particle size (PS) needed to reach the different regions of our pulmonary system. The defined appropriate amount of drug must be deposited past the oropharyngeal region in order to achieve therapeutic effectiveness.

Particles with aerodynamic particle size distribution (PSD) between one and five micrometers , sediment on the bronchial and alveolar regions of the pulmonary tree, achieve optimal pulmonary penetration and can be approved size wise for products with inhalation proposes [4].

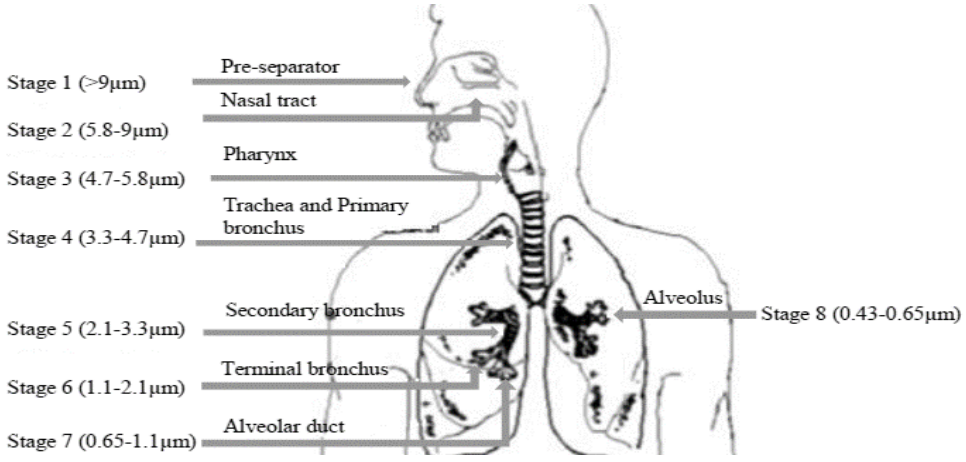


Figure 1-1 Particle size limits for inhalation drugs delivery pathways of the human respiratory (system adapted from [13]).

The performance of inhalation devices is extremely dependent of the geometric and aerodynamic PSD, of the particle shape and of the powder dispersion characteristics.

Those parameters affect the dissolution rate of the APIS, their bioavailability and the drug release rate for immediate, sustained and controlled release formulations. They influence also in vivo particle distribution and deposition, absorption rate and clearance time, affecting both content and dose uniformity.

The aerosolization behavior and performance of respiratory formulations are also highly dependent of the PSD and particle shape.

Particle size is a valuable indicator of quality and performance. As mentioned previously, powders in the range of one and five micrometers aerosolize better and penetrate deeper into the lungs than bigger particles. Therefore, it is very important to measure and control the PSD, especially on formulations intended for inhalation.

In the case of ophthalmic formulations, particle size has a major role in drug absorption. Microparticles with a mean diameter ranging from one to three micrometers are ideally for ophthalmic drug controlled release. As the drug is administered, the particles are retained in the ocular cul-de-sac and the released rate has to be adequate so the drug can successfully penetrate the ocular tissues [4].

Pharmaceutical formulations with particles bigger than 25 μm are not suitable for ophthalmic delivery as they may cause irritation to the eye. Nanoparticles without bioadhesion are also not appropriated, as they are quickly eliminated from the precorneal region.

Regarding oral dosage forms, particle size tend to range from one hundred to two hundred micrometers due to powder compaction and flow characteristics.

Interestingly, for chewable (taste-masked) and fast-disintegrating tablets, smaller particle sizes comprised between twenty and fifty micrometers are desirable as they improve the dissolution rate.

In summary, it is assumed that particle size plays a role in every step of oral dosages formulations from blending, granulation and direct compression, to coating [4].

The API particle size and shape are critical parameters in drug product formulations and API isolation conditions have a great impact on them. Depending if the APIs are milled, spray dried or lyophilized, those two properties will be controlled by the operating parameters used in the unit operations [4, 12].

1.4 Micronization

Many of today's APIs exhibit low solubility and permeability. In order to improve their solubility and bioavailability, APIs are often milled to smaller sizes.

Milling techniques are “top-down” approaches that involve the application of an external mechanical energy to physically force the coarse and broad particles to break down originating fine particles.

The comminution of particles by milling for pharmaceutical industry proposes, can be divided into two main categories, dry milling (jet milling) and wet milling.

Each category has advantages and disadvantages, for example, jet milling can achieve micron size particles with no agglomeration but can introduce amorphous content into the APIs. Wet milling on the other hand is safer for handling potent compounds but is also associated with particle aggregation and/or difficult isolation [14].

In Table 1-2 is represented a summary of advantages and disadvantages of each milling technique.

Table 1-2 Advantages and disadvantages of different milling techniques [12, 14].

Jet Milling	**Wet Milling**
Advantages	
Faster No agglomeration Cheaper	Highly robust Reproducible process Capable of particle size fine tuning Particles with smoother surfaces Minimize polymorphic form interchange
Disadvantages	
Process control difficulties Particle morphology variability Amorphization or polymorphic forms interchange Not all products are easily jet milled.	More time consuming More expensive Not a performance enhancer to all powders (case by case evaluation) Prone to physical instability phenomena.

At Hovione, the choice of method for milling APIs depends on client’s request and / or API properties.

1.4.1. Jet milling

In jet milling, the unmicronized API powder is injected in the milling chamber through a venturi feed. The material is accelerated in the chamber by rotation and compress gas expansion. The particles that move faster collide with slower particles, causing friction due to violent particle-to-particle impacts. The milled particles are aspirated spirally and exit to a collection chamber by the central outlet.

Bigger particles that are not able to escape by the central outlet, return to the milling zone where they are further micronized. Meanwhile other finer particles leave the chamber, carried out by the space gas to be collected [12].

Figure 1-2 represents a conventional jet milling process.

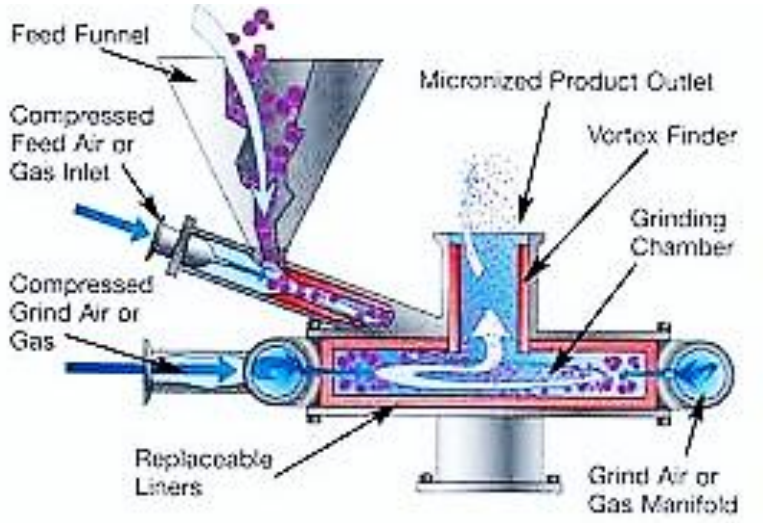


Figure 1-2 Schematic showing principles of operation of a jet miller (adapted from [15]).

1.4.2. Wet milling

A wet milling process has broad range of applications. Cell rupture, dispersions, emulsions and particle size reduction are examples of pharmaceutical applications of this process.

Wet milling is a process of milling where the size reduction of particles are suspended in a liquid medium. Wet milling is particularly suited for APIs with a high residual moisture content (>50% moisture) because dry milling may be problematic for these APIs.

Wet Polishing (WP) can be viewed as a wet milling technique capable of achieving micron and sub-micron particle sizes. It is the combination of a wet-based particle size reduction technology with spray drying as an isolation step. This step improves product stability and provides a minimization of possible hydrolytic degradation of the API [12, 14].

In a top-down approach, a high pressure homogenization is the first step of a wet polishing process and consists in forcing the API suspended in an anti-solvent media, through very small milling cameras under very high pressures. This enormous pressure drop causes cavitation, high shear forces and particle-particle collisions are the main driving forces for particle size reduction.

Proper selection of operation parameters (pressure, nozzle size, flow configuration, number of passes) and process parameters (anti-solvent media, solid content) can yield very narrow PSD [14].

1.5 Spray Dryer

Spray drying is a technique that is employed to isolate a powder from a solution, suspension or emulsion. Spray drying has the advantage of being a single-step operation, which involves atomization, drying and solvent vapors continuous separation. [16].

In the case of wet polishing, spray drying is used to isolate the micronized API after a wet milling process [17].

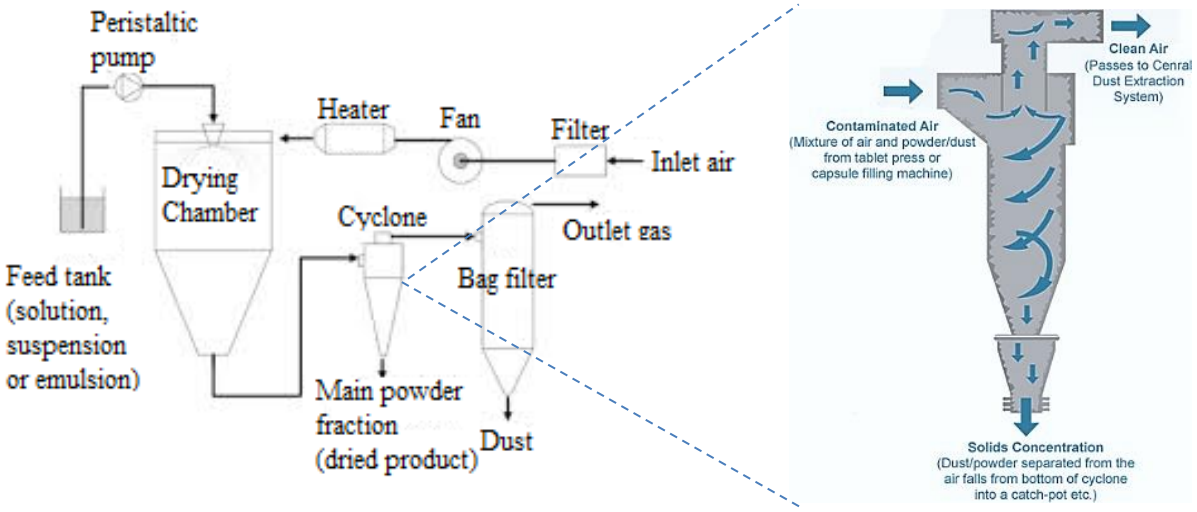


Figure 1-3 Schematic diagram of the small-scale spray dryer. (adapted from [18]).

The feed atomization generates small droplets and subsequently vaporization of the solvent takes place inside the drying chamber. The resulting dried powder enter a cyclone where the dried power fraction is collected in a powder collector placed at the bottom of the cyclone. At the same time, drying gas, solvent and fine particles exit the cyclone by the top outlet into a filter bag before exhausting. Spray drying allows a high precision control on particle properties and is a straightforward process of solvent removal from of solutions and suspensions [16].

1.6 Particle size analysis

The most critical parameter of particle characterization is particle size. This property affects a range of product characteristics such as reactivity, stability, efficacy of delivery, texture, appearance flowability, viscosity, packing, density between others.

Being a particle a three-dimensional object and, in most case scenarios, having a shape very different from a perfect sphere, it becomes impossible to characterize them by a single dimension. The most often solution to this problem is to think of particle size as a concept of equivalent sphere. With this in mind, the characterization technique must be careful considered. By measuring different properties of a particle (max. length, min. length, volume, surface area etc.) we can only compare measurements on a powder using a technique with the same equivalent sphere model [19, 20].

The Figure 1-4 shows some of the different answers possible for a single grain of sand.

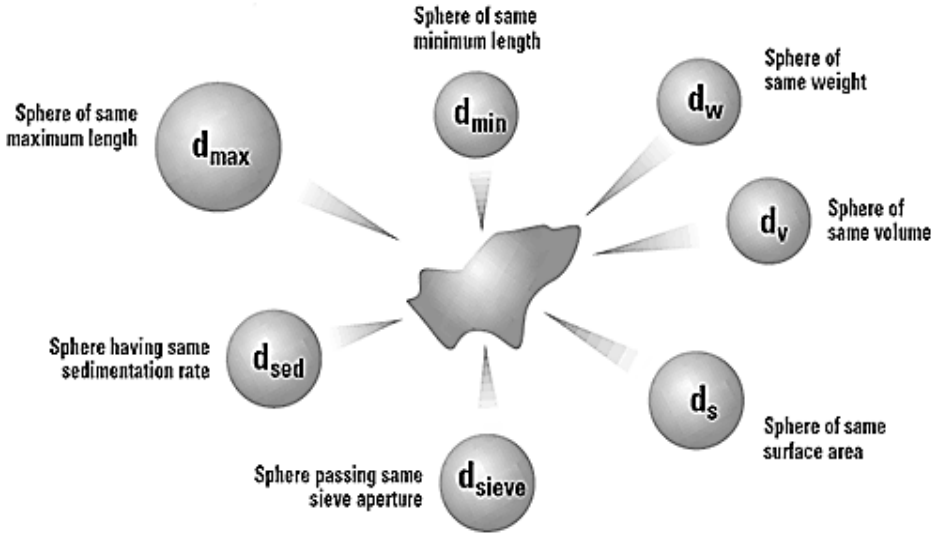


Figure 1-4 Concept of equivalente spheres (adapted from [19]).

As for the data result, the particulate sample can be represented in diverse ways or types of distributions, for example: weighted, number weighted, volume weighted or intensity weighted. The sample to analyze will consist of a statistic distribution of particles of different sizes that is usually represented in the form of a frequency distribution curve or a cumulative distribution curve.

The volume weighted distribution it is the most common distribution used especially in techniques of laser diffraction. The data results are reported as percentiles parameters based upon the maximum particle size for a given percentage volume of the sample. The most common percentiles reported are Dv10, Dv50 and Dv90, where D stands for diameter and v stands for volume-weighted distribution [19, 20].

The percentiles described above will be written as d(0.1), d(0.5) and d(0.9) and are described in Figure 1-5.

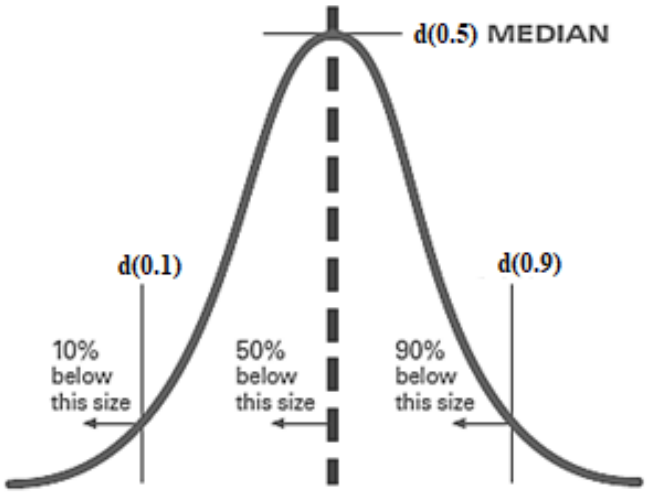


Figure 1-5 Particlie size distribution curve presenting d(0.1), d(0.5) and d(0.9) (adapted from [20]).

The span is a PSD parameter that can be calculated using the percentiles d(0.1), d(0.5) and d(0.9). The span indicates if the PSD is wide or narrow. Typically, a PSD with a high span value denotes the presence of two distinct populations: a population of fines and other of larger or agglomerated particles. Usually, a normal (Gaussian) distribution has a span between one and two [20].

Span is calculated by Equation 1-1- Span equation.

$$\text{Span} = \frac{\text{d}(0.1) - \text{d}(0.9)}{\text{d}(0.5)}$$

Equation 1-1- Span equation (Addapated from [20]).

For a corrected and accurate assessment of product analysis, it is necessary to evaluate all possible parameters such as particle size and shape, surface and mechanical properties[19, 20].

The techniques for particle size determinations carried out for this thesis were laser diffraction techniques from two different manufacturers Malvern (Malvern Mastersizer Hydro 2000S and Mastersizer 3000 Hydro MV) and Sympatec (Sympatec Helos, Rodos/M and Aspiros). Additionally, scanning electron microscopy was performed to confirm the validity of the results obtained by laser diffraction techniques.

The first step in every method of particle size analysis using laser diffraction is an analysis by microscopy as stated in the European Pharmacopeia: “inspect the sample to be analyzed, visually or with the aid of a microscope, to estimate it size range and particle shape”. The technique of microscopy used in this study is Scanning Electron Microscope as mentioned and describe in detail in the sub-chapter 1.6.1 [21].

1.6.1 Scanning Electron Microscope (SEM)

A Scanning Electron Microscope (SEM) is a magnification equipment where a beam of high-energy electrons interacts with solid-state samples. This process generate signals that provide information about the sample in the form of three-dimensional images, such as external morphology, chemical composition and orientation of the different particles in the sample. The electrons are generated by an electron gun placed at the top of the microscope column. Electrons are produced by heating a filament that is often made of tungsten and has a structure that ensures that a very narrow stream of electrons is emitted. The electrons generated by the electron source are accelerated through the microscope column that is placed under vacuum so there are any interaction of the atoms and molecules presents in the column with the electron beam. This acceleration in given by an anode, which is negatively charged, and repels the negatively charged electrons, causing them to accelerate.

Magnetics lenses allow the electrons to focus onto the sample and ensure that only a narrow beam of electrons hits the sample. Afterwards the sample scatters the electrons and the amount of scattering is highly dependent on factors such as the sample height, chemistry, and three-dimensional structure. Additionally, secondary x-rays are emitted due to electron-sample atoms interactions. Since every atom has a unique atomic structure, this phenomenon is used to identify which elements are present in the sample. This technique is known as Energy-Dispersive Spectroscopy (EDS) [22, 23].

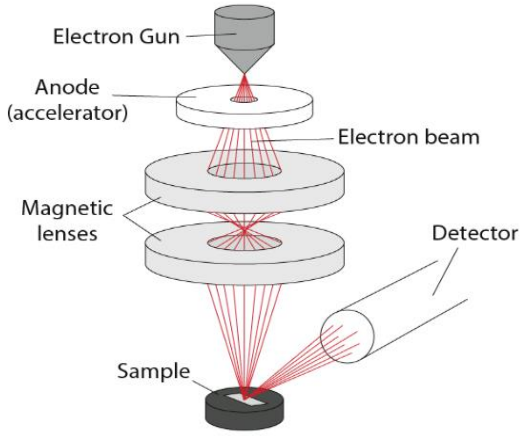


Figure 1-6 Schematic image of SEM tecnique (adapted from [22]).

1.6.2 Laser Diffraction

Laser diffraction is a widely used particle sizing technique that determines PSDs by measuring the angular variation in the intensity of light scattered as a laser beam passes through a dispersed particulate sample. Large particles will scatter light at small angles relative to the laser beam and small particles will scatter light at wider angles [19]. To simplify the primary function of a laser diffraction instrument is to record angle and intensity of scattered light.

A laser diffraction instrument consists of at least one light source with high intensity and monochromatic, a sample handling system to control the interaction between particles and incident light, and at last a set of high quality photodiodes to detect the scattered light by the particulate sample.

The data resulting from the photodiodes detection is then processed by an algorithm that consists of an optical model with the mathematical iterations necessary to generate the data from the scattered light in the form a PSD [20].

There are two optical models implemented in laser diffraction, the Fraunhofer Approximation and the Mie scattering Theory that are described in the sub-chapter 1.6.2.1.

1.6.2.1. Optical models

In the early times of laser diffraction, the Fraunhofer Approximation was widely used, being still popular in older laser diffraction instruments. This model bases its theory on certain assumptions to simplify the calculation. In this model the particles are assumed to be spherical and opaque, scatter equivalently at both wide and narrow angles and interact with light in a different manner than the medium they are in.

Rapidly these assumptions became limitations leading to a discredit of the Fraunhofer Approximation where measurement estimation accuracy for particles below approximately twenty microns in size were considered unacceptable.

Gustav Mie developed and proposed a similar solution to Maxwell's electromagnetic equations for scattering from spheres.

This solution addressed Fraunhofer Approximation limitations improving sensitivity to smaller particle sizes, a wide range of opacity. In this model, the analyst needs only to input the refractive index of particle and of the dispersing medium. However, Mie theory model also makes assumptions. It assumes that the particle is spherical and that the refractive index of particle and surrounding medium is known. [19, 20].

1.6.2.2. Sample dispersion

In laser diffraction techniques is mandatory that the sample is dispersed in a way that each particle is isolated from other particles. There are two types of dispersion techniques: a wet dispersion, where the particles are dispersed in a liquid medium, and a dry dispersion, where particles are dispersed in a gas, usually air.

For the wet dispersion, the individual particles are suspended in a dispersant. The dispersant lowers the surface energy of the particles leading to a reduction of particle-particle attraction forces, allowing them to be separated in suspension.

For the dispersion to be more effective it is usual to apply some external energy to the sample by stirring or agitation and ultrasonic irradiation for very fine materials or agglomerates.

Dispersants with high surface tension such as water, highly benefit from the addition of a small amount of surfactant to improve the wetting of the product.

As for dry powder dispersion, the dispersant is a gas stream, most typically clean dry air. This is a higher energy process than wet dispersion and there are three different types of dispersion mechanisms. These mechanisms are displayed in Figure 1-7 ordered by magnitude of energy involved.

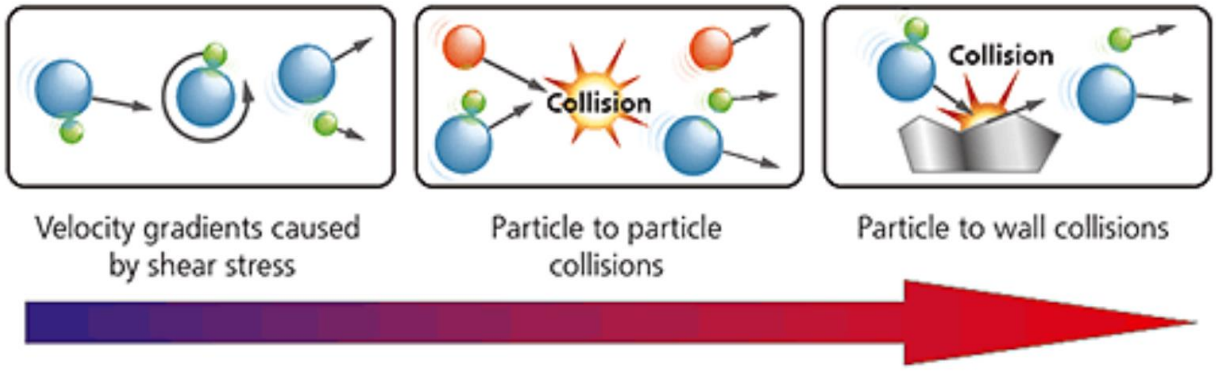


Figure 1-7 Dry powder dispersion mechanisms with increasing energy/ aggressivity (adapted from [19]).

Dry dispersion is not recommended for very fine powders (< one micron) because the particle-particle forces of attraction in these materials are very difficult to overcome. For fragile particles, lower energy should be applied to ensure that particles are not broken during the dispersion process [19].

2. Equipment and Materials

All the equipment used were property of Hovione's analytical R&D Products laboratory.

During the course of this thesis, the product samples were analyzed by scanning electron microscopy and, whenever it was possible, analyzed by both wet and dry dispersion methods.

The products classified as potent (Occupational Exposure Limit (OEL) 0.03-10 um/m^3) by Hovione's internal procedures were manipulated according to Hovione's Products Handling Requirements Matrix [24].

2.1 Scanning Electron Microscopy

As stated in chapter 1, prior to any PSD analysis, it is recommended to perform a SEM analysis.

The SEM equipment used was the Desktop Scanning Electron Microscope Phenom ProX Generation 5.

Figure 2-1 Desktop scanning electron microscope (SEM) Phenom ProX Generation 5 (adapted from [25]).

The SEM preparation method is simple and require three steps.

The first one is the sample preparation that must take place in a contained environment accordingly to category of the product to be analyzed. This preparation consists in placing a product sample on a carbon double sided adhesive tape previously mounted on a clean stub pin.

The second step is to remove the excess powder that did not adhered to the carbon tape, by aspirating the pin using HEPA filtered vacuum. This aspirating step also allows the analysis of suspensions as it remove and dries the excess of liquid medium, enabling the analysis of a dry powder sample.

The last step is to place the sampled pin on the sample holder. For a proper analysis is necessary to rotate and lower the sample until two millimeters below the top surface (4 vertical marks) before placing it inside the equipment.

Figure 2-2 a) Phenom sample holder and stub pin ; b) Phenom sample preparation set.

2.2 Laser diffraction by wet dispersion

For the wet dispersion laser diffraction method the first equipment used for the PSD analysis was a Malvern Mastersizer Hydro 2000 S.

This equipment combines two separated units: a laser unit (Mastersizer) and a dispersion unit (Hydro 2000 S). The laser unit stays the same can be combined with other dispersion units depending on the type of analysis to be done.

Figure 2-3 Malvern Mastersizer Hydro 2000 S unit (adapted from [26]).

The mentioned unit combination enables the measurement of the PSD since it allows the laser beam of the laser unit to pass through the dispersed particulate sample on the measurement cell of the dispersion unit.

For known products, the sample preparation and the technique are usually described in a previous validated method stored in intranet.

For a new product analysis, a new method must be created based on the guidelines described in Hovione's Corporate Operating Procedure (COP037) [27, 28].

After the SEM analysis the first step is to choose an appropriate dispersant. In Table 2-1 are presented the ideal properties of a good dispersant [1].

Table 2-1 Ideal characteristics of a good disperant medium [1].

Ideal characteristics of a good dispersant
• Provide good wetting of the sample (enabling dispersion) • Do not dissolve the sample • Do not contain bubbles • Have a suitable viscosity • Be transparent to the laser beam • Have a different refractive index of the sample • Be chemically compatible with the materials used in the instrument

A list of possible dispersants ordered by decreasing order of polarity is shown in Table 2-2.

Table 2-2 Dispersants ordered by decreasing order of polarity (adapted from [1]).

Dispersant	**Polarity**
Water Organic acids Alcohols (methanol/ethanol/isopropyl alcohol) Simple alkanes (hexane/ heptane/iso-octane/cyclohexane	Polar ⬇ Non-Polar

Wetting efficiency depends on the surface tension between the particles and the liquid. A good wetting between particulate sample and dispersant presents an uniform suspension of particles in the liquid. In opposition, poor wetting may present droplets of liquid on top of the powder or significant agglomeration and sedimentation.

The wetting process can be improved by adding a surfactant to reduce the surface tension. The surfactants are chosen based on the polarity the dispersants available. In Table 2-3 are presented several combinations of dispersants and surfactants used in product analysis.

Table 2-3 Standard combinations of dispersants and surfactants (adapted from [1]).

Dispersant	**Surfactant**
Water Methanol Isopropanol	Tween 80/20, SDS, IGEPAL
N-hexane Heptane Isoparaffin	Span 85, Lecithin

The next sept is to decide the sample amount. Usually an appropriate amount is 30 to 50 mg, being the lower limit 20 mg. At least half of the suspension must be used to be considered representative of the sample particulate.

After the dispersant/surfactant and the sample amount selection, is necessary to define the default measurement conditions to get a robust result from the laser diffraction measurement. These include obscuration range, measurement duration and stirring speed.

The sample concentration in a laser diffraction technique is measured by a parameter called obscuration, which is the percentage loss of laser light through the sample. This parameter may lead to a multiple scattering effect. Fine particles are more easily affected by this phenomenon while coarser particles are more likely to be affected by sampling [1].

The multiple scattering phenomenon can happen if the obscuration range is not the appropriated. In Table 2-4 are presented the recommended obscuration ranges depending on the particle size.

Table 2-4 Recommended obscuration ranges for each particle size range (adapted from [1]).

Particle size	Obscuration range
Fine particles	5 to 10% (less than 5% may be required for <1μm)
Coarse particles	5 to 12%
Polydisperse samples	15 to 20%

Another condition to be selected is stirring. The stirring must ensure that the dispersion is homogenous and that the sample passing through the measurement cell is representative.

Stir speed titration must be performed to ensure that a good suspension is obtain.

One of the most important analytical condition is the ultrasounds (US). It necessary to perform US titration curves for different values of US, using independent samples. This selection it is only considered satisfactory if the obscuration does not decrease during the analysis.

For the analysis to be considered valid the obscuration value needs to stay within the ranging set and the residual value must be inferior to two, as stated in Hovione's Corporate Operating Procedure (COP037).

The last step was to perform stability and repeatability tests, evaluating when a plateau in the PSD parameters is reached and while the obscuration remains constant.

The angular scattering intensity data is then analyzed to calculate the size of the particles that created the scattering pattern using the Mie theory of light scattering. The particle size is reported as a volume equivalent sphere diameter.

Mie theory of light scattering requires knowledge of the optical properties of both the sample and the refractive index of the dispersant. For samples where the optical properties are not known, the user can either measure them or estimate them using an iterative approach based on the fit between the modeled data and the sample actual data [1, 29].

The second equipment used was Mastersizer 3000, which is an upgraded version of Mastersizer 2000. This equipment is the latest generation of particle sizing instruments. It has a completely new optical core design and delivers fast measurement times for high sample throughput.

A typical Malvern Mastersizer 3000 system is made up of three main elements: an optical bench, a sample dispersion unit and an instrument software. A dispersed sample passes though the measurement area of the optical bench, where a laser beam illuminates the particles. A series of detectors then accurately measure the intensity of light scattered by the particles over a wide range of angles. The sample dispersion is controlled by a range of wet and dry dispersion units, ensuring that the particles are delivered to the measurement area at the correct concentration and in a suitable, stable state of dispersion.

The instrument software controls the equipment during the measurement process and analyzes the scattering data generating a PSD. It also provides instant feedback during method development and an advice on the quality of the results [30].

Figure 2-4 a) Malvern Mastersizer 3000 equipemente; b) Hydro MV unit (adapted from [30]).

In the studies carried out for this thesis the sample dispersion unit used was the Hydro MV which is a medium volume unit for the controlled, automated wet dispersion of samples for particle size analysis. Designed for applications that require smaller sample sizes, the Hydro MV is especially valuable when the supply of test material is limited or when dispersant usage must be minimized [30].

The method development and validation for this equipment follows the steps previously described for Malvern 2000.

2.3 Laser diffraction by dry dispersion

Laser diffraction by dry dispersion The Sympatec equipment is composed of several independent modules according to the clients' needs.

In Figure 2-5 is represented the Sympatec equipment unit with the available modules setting used in these studies for dry dispersion analysis.

Figure 2-5 Sympatec equipment module stetting with a) ASPIROS; b) RODOS/M and c) HELOS.

The main component of the Sympatec unit is the HELOS (Helium-Neon Laser Optical System) module. This module contains the laser and its measuring ranges depend on which one of the four lenses is used. In Table 2-5 is described the range of each four lens available [31].

Table 2-5 Range set for each lens (adapted from [31]).

Lens	Range
R1	0.18 µm – 35 µm
R2	0.45 µm – 87.5 µm
R4	1.80 µm – 350 µm
R5	4.50 µm – 875 µm

The RODOS/M is the dry dispersion unit and is attached to HELOS module. It can be used for almost all types of dry powders with a PS range between 0.1µm and 3500µm. This module is fully automatic as the analytical parameters are controlled by the software (WINDOX v5.9.1.2) [31].

This unit uses compressed air to disperse the sample particles, generating a dry aerosol that passes in front of the laser beam. Once the sample is analyzed, the powders are collected by suction into a HEPA filtered vacuum [32]. ASPIROS accessory was used as it is safer to work with potent products. Vials are filled in a LEV with a few milligrams of the product, capped and placed inside this accessory, minimizing the risk of exposure [33].

Figure 2-6 Sympatec vial ready for analysis [34].

Sympatec uses the Philips-Twomey algorithm as a mathematical algorithm model [35].

In these studies, the HELOS unit laser evaluated the PSD according to FREE (Fraunhofer Enhanced Evaluation) parameter that applies the Fraunhofer theory down to 0.1μm particles. This theory is possible to be applied without knowledge of optical parameters of the sample.

It is important to mention that the FREE is different from the Fraunhofer theory, since it is the "upgraded" version with the Philips-Twomey algorithm, which is meant to circumvent the limitations associated with the Fraunhofer theory [31].

During method development using this equipment the variables to consider are pressure of the dispersing media (air), feed rate velocity on the ASPIROS module and density of the product.

The fists step is to do a SEM analysis and based on the information collected, an appropriate lens selected

Only the pressure and the feed rate velocity variables were evaluated during Sympatec's method development. Generally, the density of the products is unknown, therefore a standard value of 0.3 g/cm3 was used for all the products analyzed.

Sample preparation is simple and consists of filling the glass vial with some milligrams of the powder sample. The quantity varies with the product particle size but is recommended to be enough to obtain at five to fifteen percent of optical concentration.

The final step of method development is to perform several titration curves of feeding rate velocity and pressure that are able to provide a good dispersion without damaging the particles.

3. Methods

In this chapter are described two performed laser diffraction method developments, for wet dispersion and dry dispersion techniques.

For the wet dispersion method, the equipment used was Malvern 3000 Hydro MV and for the dry dispersion, the equipment used was Sympatec.

3.1. Wet dispersion method development for Malvern 3000

The previous analytical method for [IH11] PSD analysis was performed using Malvern 2000. Due to performance evaluation and for analysis simplification, it was decide to do a method development for [IH11] PSD analysis in Malvern 3000.

The analytical conditions for the previous Malvern 2000 method for [H11c] solid powders are presented on the Table 3-1

Table 3-1 Analytical conditions for the PSD method on Malvern 2000 for [IH11c]

Settings	Samples
Accessory	
Sample handling unit	Hydro 2000S
Stirrer speed (rpm)	2100
Ultrasonic amplitude (%)	The amplitude is the one that will correspond to 66 +/- 5 V
Time US (min)	01:00
Measurement options	
Material	
Sample material name	IH11
Refractive index	1.52
Absorption	0.01
Dispersant name1)	Acetone
Refractive index of the dispersing agent	1.36
Result calculation	
Model	General purpose
Calculation sensitivity	Normal sensitivity
Particle shape	Irregular
Measurement	
Sample measurement time	10 seconds (10000 snaps)
Background measurement time	10 seconds (10000 snaps)
Number of measurements	3
Delay	10 seconds
Average	Create average result
Obscuration limits	5 – 11%

To begin the Malvern 3000 development method, some conditions were maintained such as the dispersant medium, the refractive index and the absorption index.

The first step to the method development was to perform ultrasound curves to understand the product behavior and if the default conditions were appropriate for the analysis. The default conditions are shown in Table 3-2.

Table 3-2 Default conditions for ultrasound curves [IH11c] method development

Settings	Samples
Accessory	
Sample handling unit	Hydro 2000S
Stirrer speed (rpm)	2100
Ultrasonic amplitude (%) ,(unit US)	First Variable, V
Time US (min)	Fist Variable
Measurement options	
Material	
Sample material name	IH11
Refractive index	1.52
Absorption	0.01
Dispersant name	Acetone
Refractive index of the dispersing agent	1.36
Result calculation	
Model	General purpose
Calculation sensitivity	Normal sensitivity
Particle shape	Irregular
Measurement	
Sample measurement time	10 seconds (10000 snaps)
Background measurement time	10 seconds (10000 snaps)
Number of measurements	3
Delay	10 seconds
Average	Create average result
Obscuration limits	5 – 11%

The first curve was performed with 40% US out of 100 maximum percentage and is represented in Figure 3-1. The product was analyzed every 30 seconds until 300 seconds total.

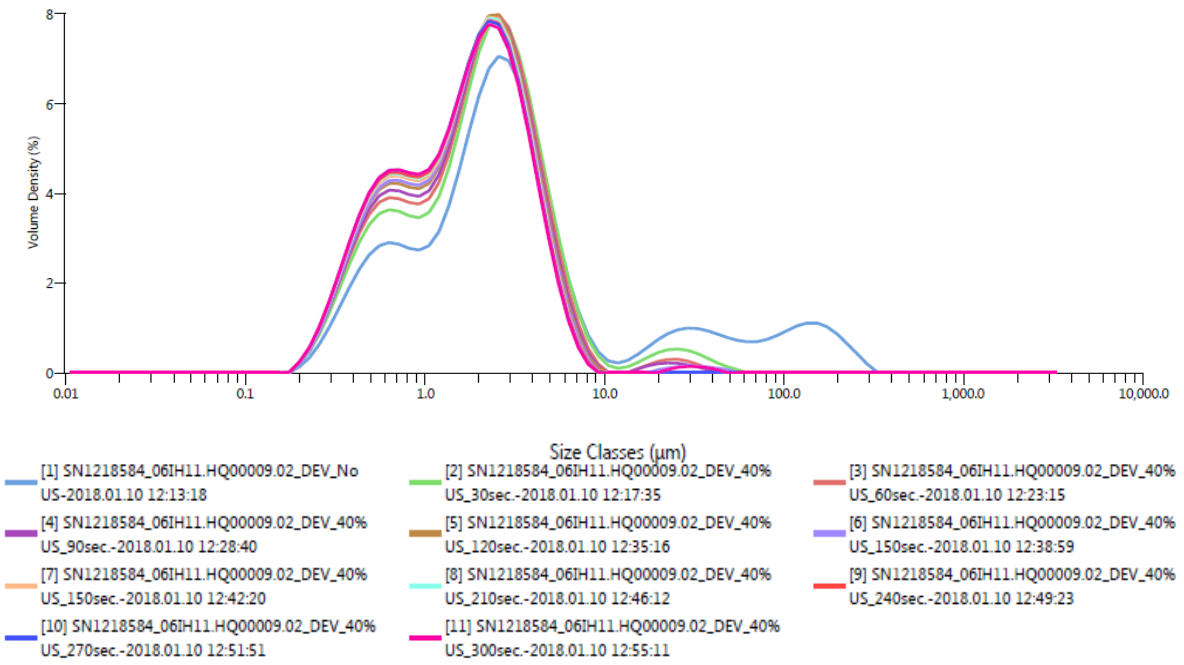


Figure 3-1 Ultrasound curve at 40% for a IH11c sample during 300 seconds

The results obtained show that the parameters d(0.1), d(0.5) and d(0.9) decreased along the ultrasound curve and show instability during the measurements.

The curve in Figure 3-2 was performed at 50% US out of 100 maximum percentage.

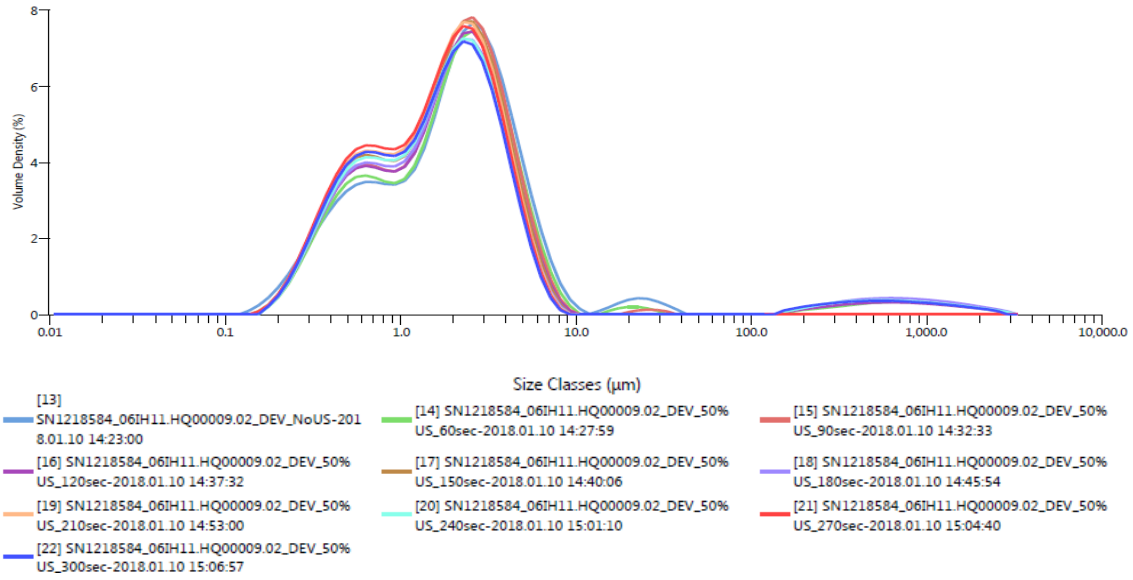


Figure 3-2 Ultrasound curve at 50% for a IH11c sample during 300 seconds.

The results obtained show a decrease in the d(0.1), d(0.5) and d(0.9) values along the ultrasound curve. It is also possible to see four PSD, which indicates sample instability.

After evaluating both curves, it was decided to increase the stirring speed to evaluate if the sample was more stable at 3500 rpm.

The curve presented in Figure 3-3 was performed at 50% US at 3500 rpm, after 300 seconds in the dispersant unit.

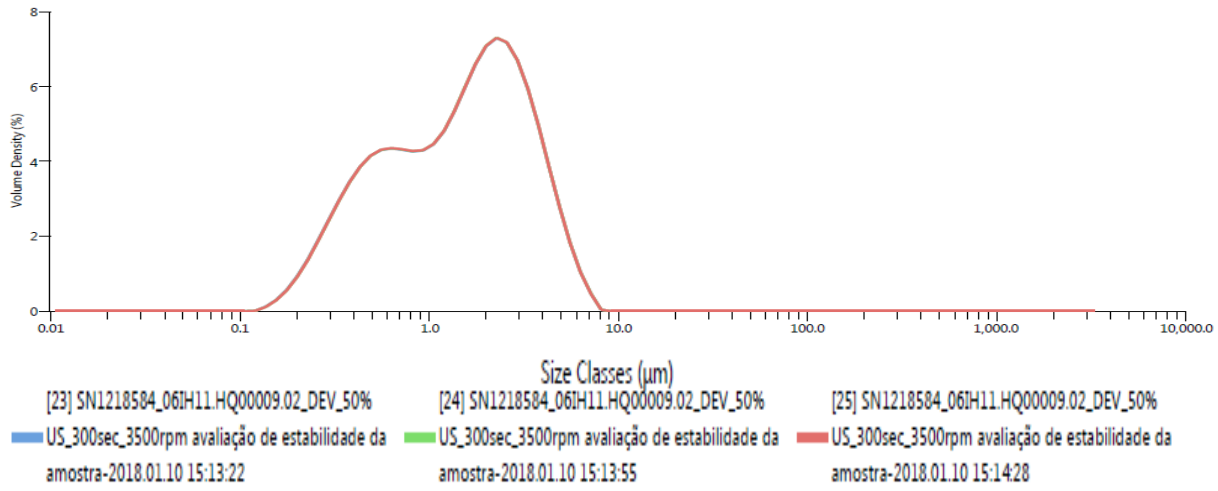


Figure 3-3 Curve at 50% after 300 seconds with a stirring speed of 3500 rpm.

There is a stability improvement of the sample measurements since the third distribution on the right in Figure 3-2 disappeared, so it was decided to change the stirring from 2100 to 3500 rpm. With this change, the first two curves were discarded and new curves were performed at 30%, 50% and 80% US with a stirring speed of 3500 rpm. The product was analyzed every 30 seconds until 300 seconds total.

Figure 3-4 shows the sample behavior during the 30% US curve.

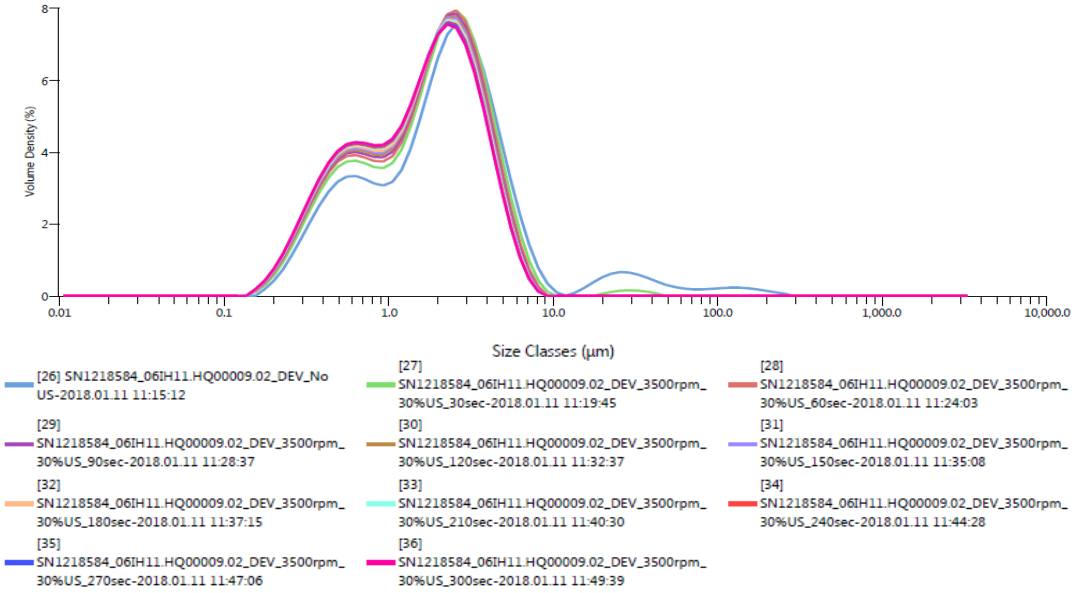


Figure 3-4 Ultrasound curve at 30% during 300 seconds with a stirring speed of 3500 rpm

The curve at 30% US showed some instability after 3 minutes of stabilization time and a decrease of the PSD parameters.

The 50% US curve is presented in Figure 3-5.

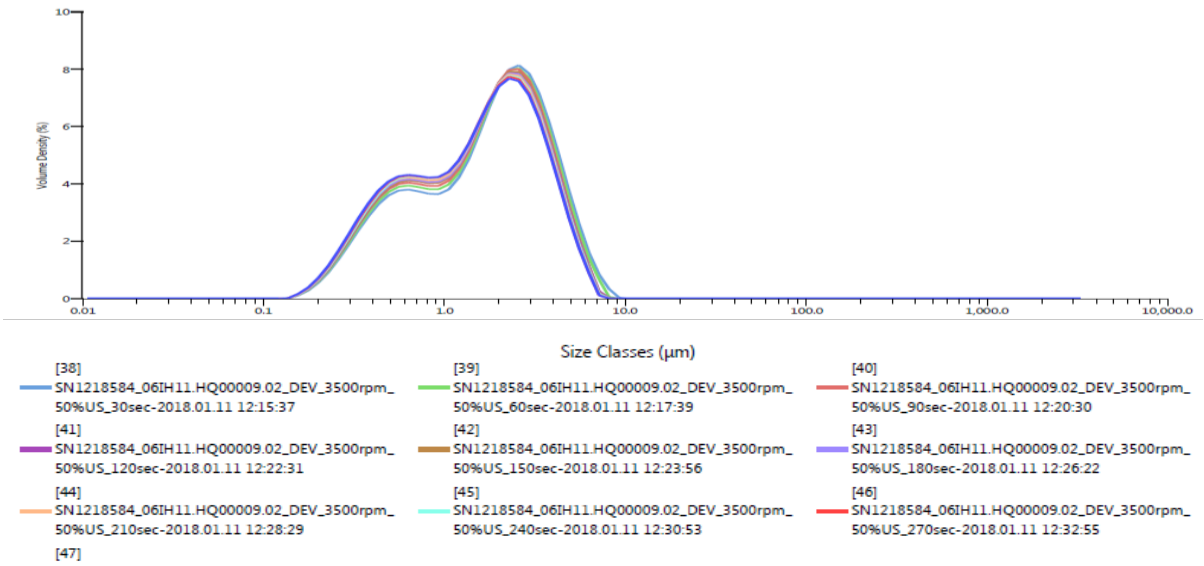


Figure 3-5 Ultrasound curve at 50% during 300 seconds with a stirring speed of 3500 rpm

The curve at 50% US showed great stabilization along the 300 seconds but there is still a decrease of the PSD parameters.

At last, the 80% US curve is described in Figure 3-6.

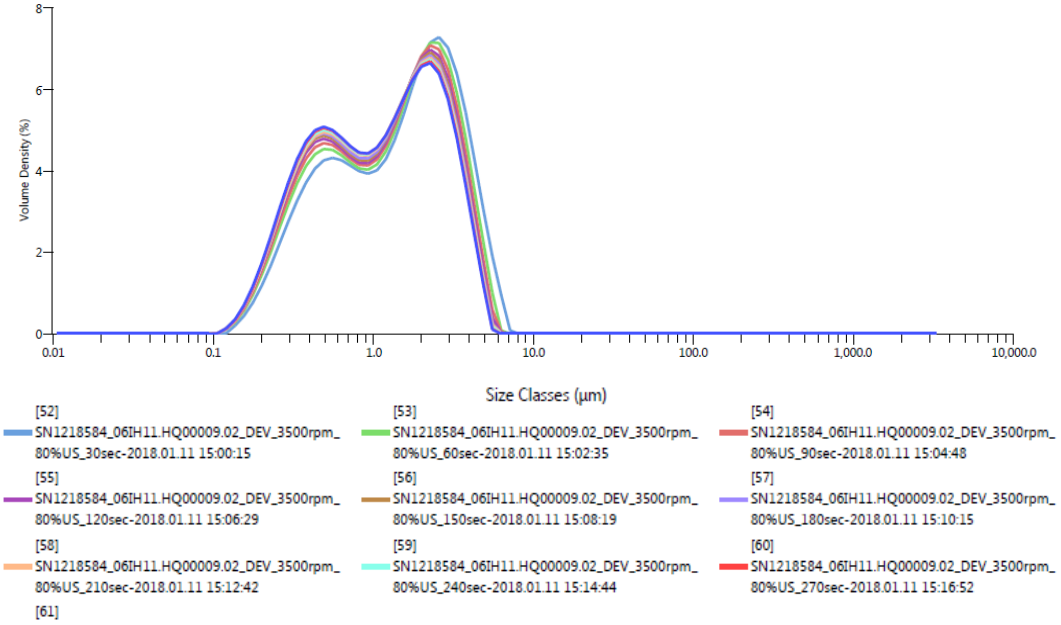


Figure 3-6 Ultrasound curve at 80% during 300 seconds with a stirring speed of 3500 rpm

The curve at 80% US showed a decrease of the PSD parameters and a good stabilization along the 300 seconds.

After comparing the three US curves it was decided to perform other two curves at lowers US percentages, at 20% and at 10%, since it was verified a decrease in the PSD parameters at higher percentages, meaning that the US % used was too high and was influencing the particle size.

The curve at 20% and 10% US are describe in Figure 3-7 and in Figure 3-8.

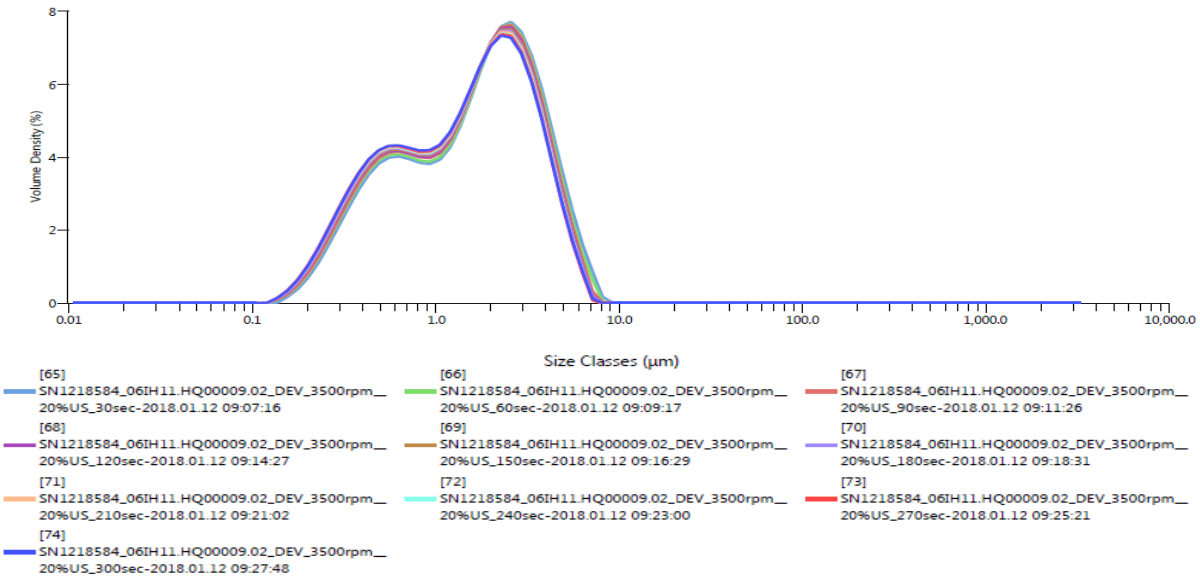


Figure 3-7 Ultrasound curve at 20% during 300 seconds with a stirring speed of 3500 rpm

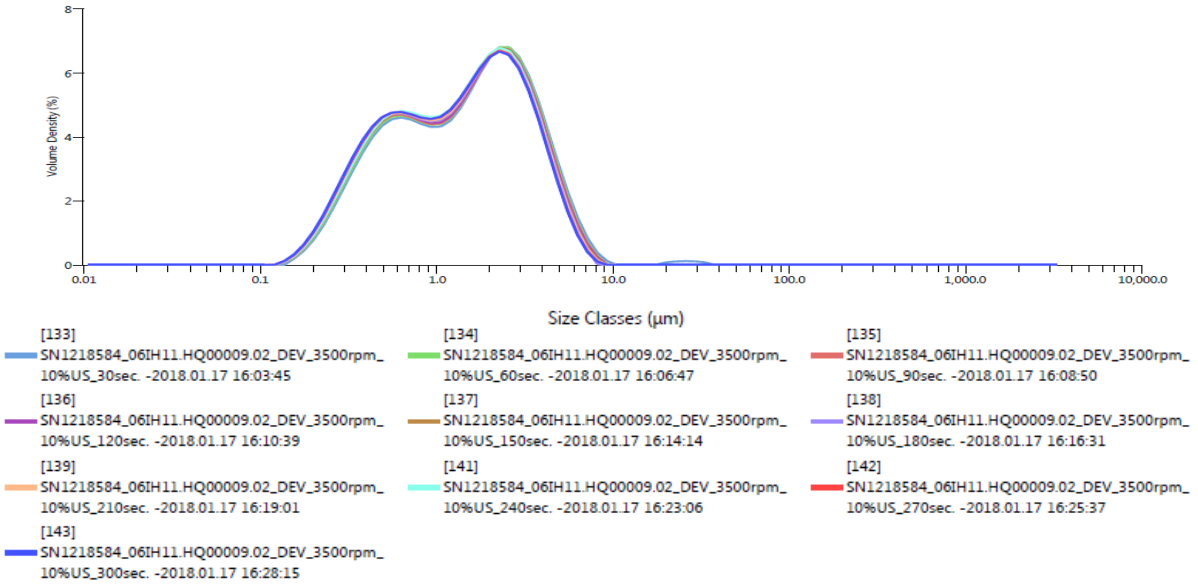


Figure 3-8 Ultrasound curve at 10% during 300 seconds with a stirring speed of 3500 rpm

By analyzing the behavior of the two curves it was decided to use 10% ultrasound. It provided a more precise curve overlay during the US application and it is always preferable to work with the minimum US percentage needed, if possible.

In order to control the left second distribution showed in every previous US curves, it was decided to change de refractive index and the absorption value of the product sample.

The curve in Figure 3-9 was performed at 10% US at 3500 rpm, whit a refractive index of 1.62 and the absorption value of 1. The product was analyzed every 30 seconds until 300 seconds total.

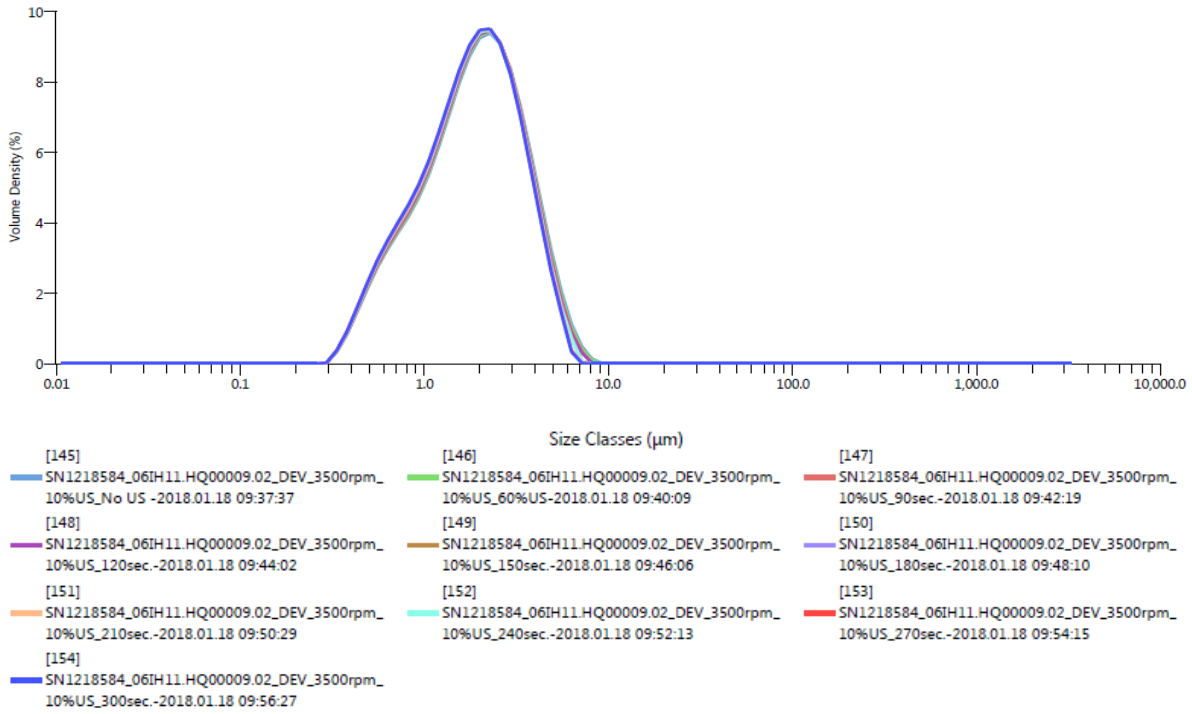


Figure 3-9 Ultrasound curve at 10% during 300 seconds with a stirring speed of 3500 rpm with diferent IR e Abs.

The second distribution to the left that appeared in Figure 3-8 is now gone and the effect on the PSD parameters is negligible. Therefore, it was decided to change of the refractive index from 1.52 to 1.62 and the absorption value from 0.01 to 1.

At this stage of the method development, the stirring speed, the US percentage, the refractive index and the absorption value are decided.

Next it was performed a repeatability test. This text consists in analyzing two samples in the same conditions and prepared in a similar way. The operational conditions are presented in Table 3-3.

Table 3-3 Analytical conditions for the repeatability and stability test on Malvern 3000 for [IH11c]

Settings	Sample(s)
Accessory	
Sample handling unit	Hydro MV
Stirrer speed (rpm)	3500 rpm
Ultrasonic (%)	10%-42V
Time of US	90 sec
Measurement options	
Material	
Sample material name	IH11
Refractive Index	1.65
Absorption	1
Dispersant name	Acetone
Refractive Index of the dispersing agent	1.36
Result calculation	
Model	General Purpose
Calculation sensitivity	Normal sensitivity
Particle shape	Irregular
Measurement	
Sample measurement time	10
Background measurement time	10
Number of measurements	3
Delay	10
Average	Create average results
Obscuration limits	5 – 10%

The curves resulting from the repeatability test at 10% US are presented in Figure 3-10.

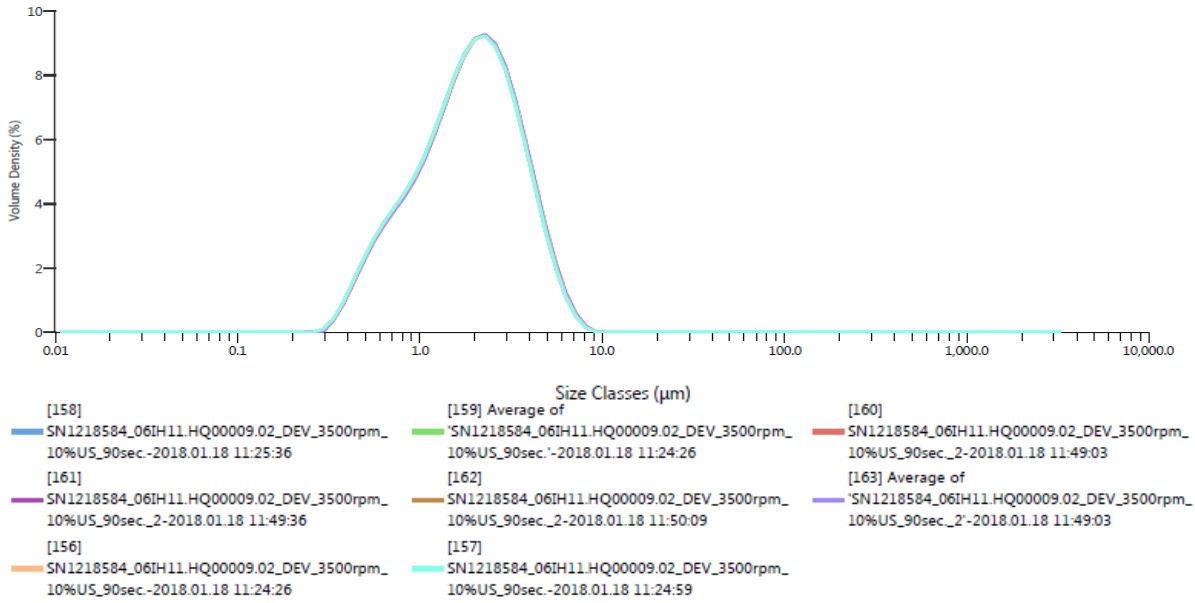


Figure 3-10 Sample 1 and Sample 2 PSD curves for the repeatability test at 10% US during 90 seconds at 3500 rpm.

The results shown a good repeatability between the samples, providing a near perfect overlay of each measurement.

Then, it was performed a stability test. This test consists in analyzing the sample PSD behavior after 5, 10, 15 and 20 minutes of analysis.

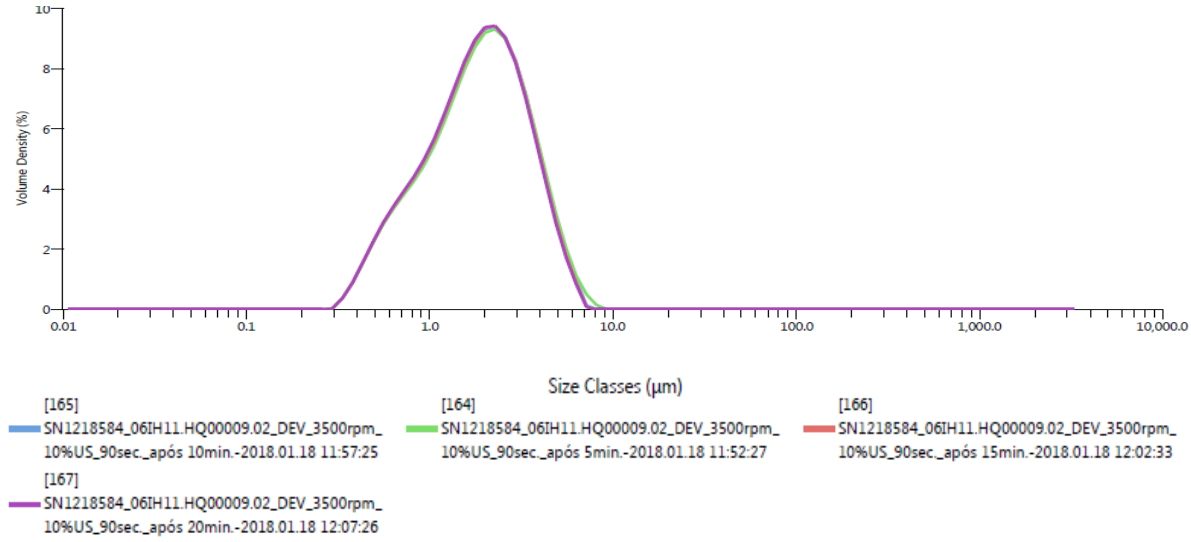


Figure 3-11 PSD curve for the stability test at 10% US during 90 seconds at 3500 rpm.

The PSD parameters (d(0.1), d(0.5), d(0.9)) slightly decreased along the stabilization time, however is was considered negligible.

The next step was to perform two more curves at 10% US with a stirring speed of 3500 rpm for [IH11c] samples, one with a bigger particle size and another with a smaller one. The goal of this final test is to confirm that the conditions chosen could be applied for different particle sizes, allowing sample analysis at every stage of the micronization process.

The results of the two curves at 10% are presented in Figure 3-12 and Figure 3-13.

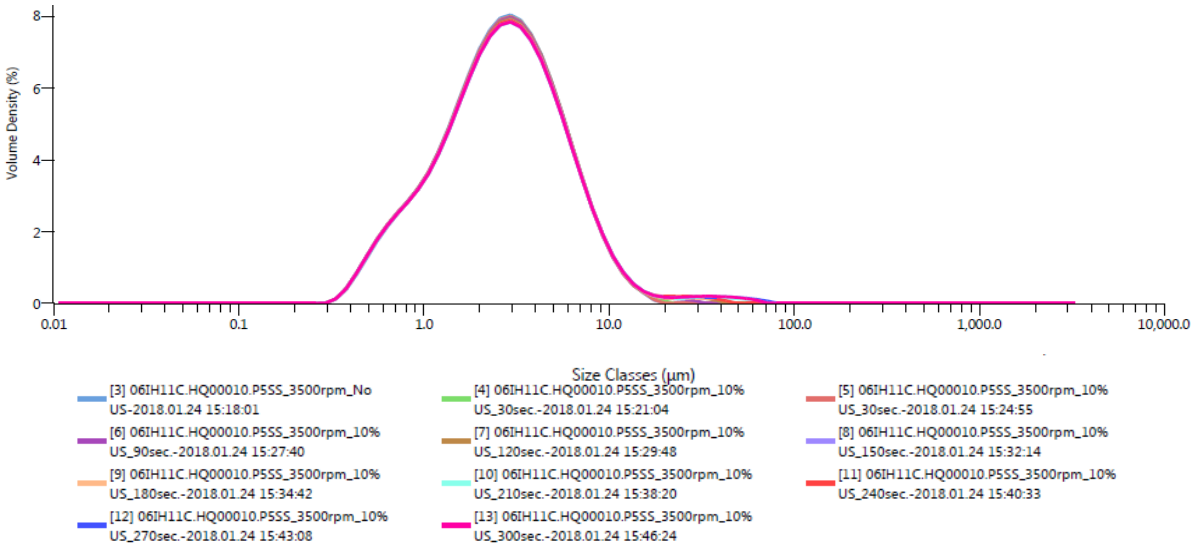


Figure 3-12 Ultrasound curve at 10% for a bigger particle size.

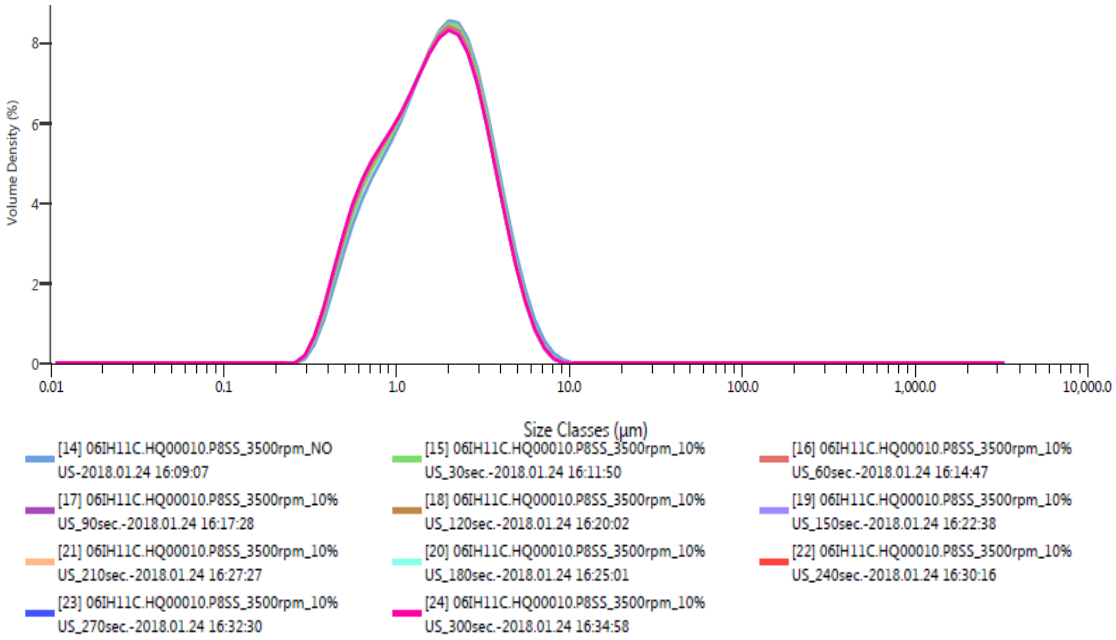


Figure 3-13 Ultrasound curve at 10% for a smaller particle size.

Both curves show a good stabilization during the 300 seconds. The curve for the bigger particle size shows a small second instability that is not significant.

In order to choose the duration of the US application it is necessary to analyze the PSD parameters behavior along the curve to see when a plateau is reach, meaning that the sample is completely de-agglomerate and that the US is not breaking the particles.

	Record Number	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	3	06IH11C.HQ00010.P5SS_3500rpm_No US	0.882	2.64	6.53
	4	06IH11C.HQ00010.P5SS_3500rpm_10%US_30sec.	0.871	2.64	6.57
	5	06IH11C.HQ00010.P5SS_3500rpm_10%US_30sec.	0.869	2.64	6.55
	6	06IH11C.HQ00010.P5SS_3500rpm_10%US_90sec.	0.870	2.65	6.62
	7	06IH11C.HQ00010.P5SS_3500rpm_10%US_120sec.	0.871	2.65	6.69
	8	06IH11C.HQ00010.P5SS_3500rpm_10%US_150sec.	0.873	2.67	6.87
	9	06IH11C.HQ00010.P5SS_3500rpm_10%US_180sec.	0.873	2.67	6.86
	10	06IH11C.HQ00010.P5SS_3500rpm_10%US_210sec.	0.874	2.67	6.90
	11	06IH11C.HQ00010.P5SS_3500rpm_10%US_240sec.	0.872	2.66	6.81
	12	06IH11C.HQ00010.P5SS_3500rpm_10%US_270sec.	0.874	2.68	6.97
	13	06IH11C.HQ00010.P5SS_3500rpm_10%US_300sec.	0.874	2.67	6.94
Mean			**0.873**	**2.66**	**6.75**
1xStd Dev			**0.00344**	**0.0146**	**0.167**
1xRSD (%)			**0.394**	**0.549**	**2.47**

Figure 3-14 PSD analytic results for the big particles sample and the selected plateau for the US time.

Figure 3-14 shows that the d(0.5) reach a plateau in the results at 150 seconds of US application, since this parameter show stability and uniformity from that point forward. For the US application time evaluation, the PSD parameter chosen was the d(0.5) since it showed a steadier behavior during analysis.

	Record Number	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	14	06IH11C.HQ00010.P8SS_3500rpm_NO US	0.638	1.73	3.89
	15	06IH11C.HQ00010.P8SS_3500rpm_10%US_30sec.	0.617	1.68	3.77
	16	06IH11C.HQ00010.P8SS_3500rpm_10%US_60sec.	0.606	1.65	3.71
	17	06IH11C.HQ00010.P8SS_3500rpm_10%US_90sec.	0.602	1.63	3.71
	18	06IH11C.HQ00010.P8SS_3500rpm_10%US_120sec.	0.599	1.63	3.70
	19	06IH11C.HQ00010.P8SS_3500rpm_10%US_150sec.	0.598	1.63	3.70
	21	06IH11C.HQ00010.P8SS_3500rpm_10%US_210sec.	0.598	1.63	3.71
	20	06IH11C.HQ00010.P8SS_3500rpm_10%US_180sec.	0.598	1.63	3.71
	22	06IH11C.HQ00010.P8SS_3500rpm_10%US_240sec.	0.598	1.63	3.71
	23	06IH11C.HQ00010.P8SS_3500rpm_10%US_270sec.	0.598	1.63	3.71
	24	06IH11C.HQ00010.P8SS_3500rpm_10%US_300sec.	0.598	1.63	3.71
Mean			**0.605**	**1.65**	**3.73**
1xStd Dev			**0.0125**	**0.0318**	**0.0553**
1xRSD (%)			**2.06**	**1.93**	**1.48**

Figure 3-15 PSD analytic results for the small particles sample and the selected plateau for the US time.

For the smaller particles, the plateau was reached for all the three PSD parameters at 150 seconds of US time as shown in Figure 3-15. Therefore, the US application time was decided at 150 seconds.

To finalize the method development it was performed one more repeatability test at 150 seconds, a stirring test and an obscuration test for validation proposes.

In Table 3-4 are presented the final conditions for the new method of PSD analysis of [IH11] samples.

Table 3-4 Final analytical conditions for Malvern 3000 analysis of [IH11c]

Settings	Sample(s)
Accessory	
Sample handling unit	Hydro MV
Stirrer speed (rpm)	3500 rpm
Ultrasonic (%)	10%-42V
Time of US	150 seconds
Measurement options	
Material	
Sample material name	IH11
Refractive Index	1.65
Absorption	1
Dispersant name	Acetone
Refractive Index of the dispersing agent	1.36
Result calculation	
Model	General Purpose
Calculation sensitivity	Normal sensitivity
Particle shape	Irregular
Measurement	
Sample measurement time	10
Background measurement time	10
Number of measurements	3
Delay	10
Average	Create average results
Obscuration limits	5 – 10%

3.2. Dry dispersion method development for Sympatec

In this section is described the development method using a dry dispersion technique for a [GD11] batch.

The first step was to analyze the SEM images presented in Figure 3-16 of the [05GD11.HQ00006].

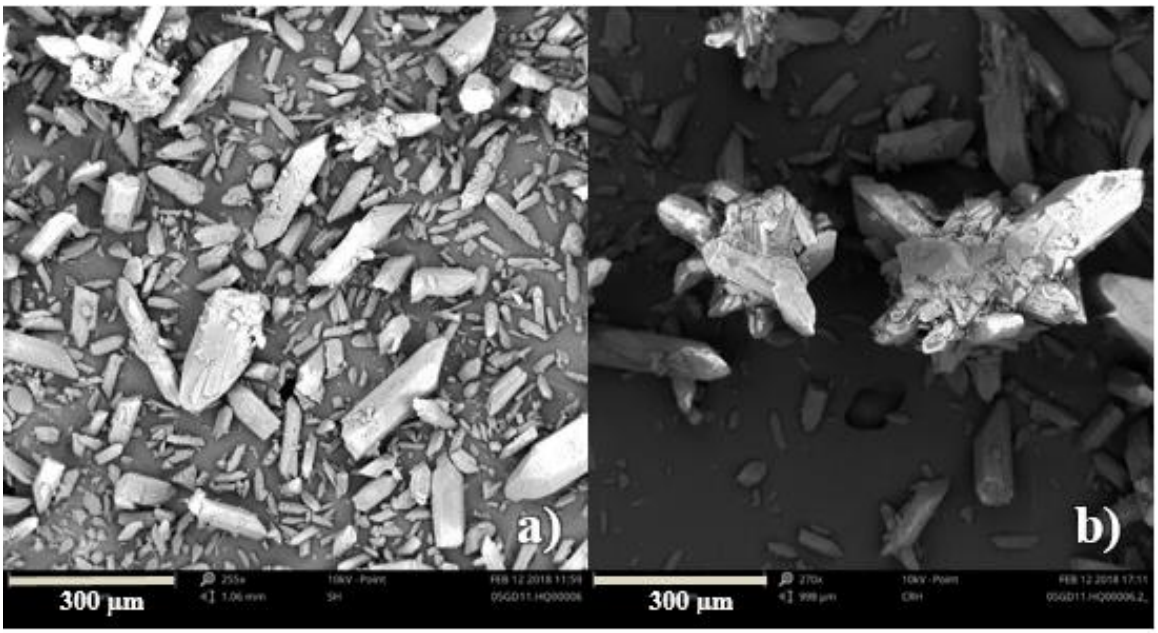
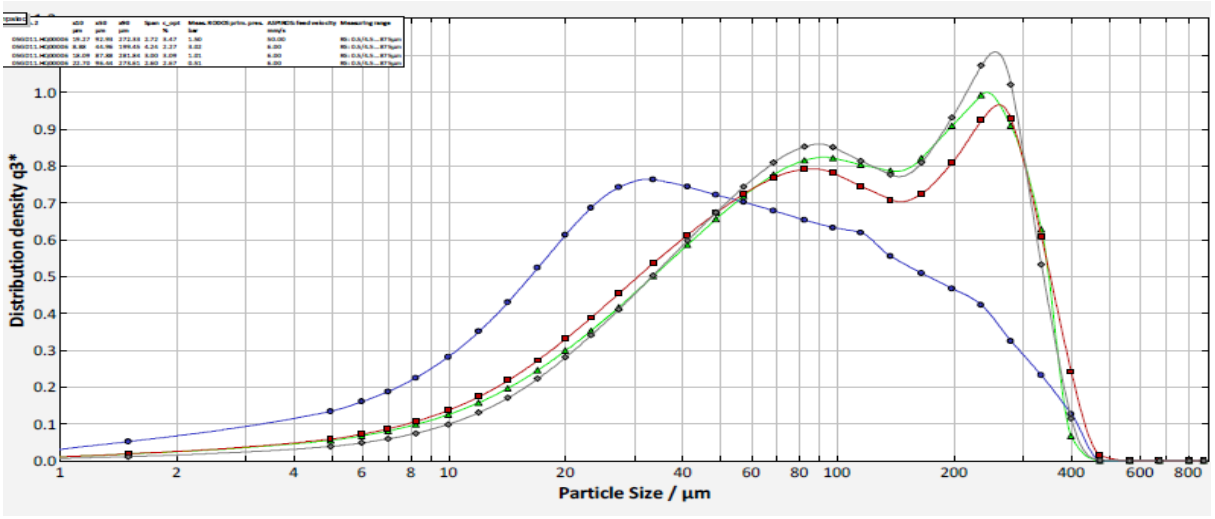


Figure 3-16 a) Powder sample of [05GD11.HQ00006] at a magnification of 255x-10kV–Point; b) Powder sample of [05GD11.HQ00006] at a magnification of 270x-10kV -Point

It is the SEM analysis that guides the Sympatec lenses selection. As it can be seen in the Figure 3-16, the overall particle size is quite large, being that the biggest agglomerates ranges from 200 to 300 µm and the smallest ranges from 7µm to 20 µm. The R5 lens is the most appropriate, since it covers a particle size range from 4.5 µm to 875 µm.

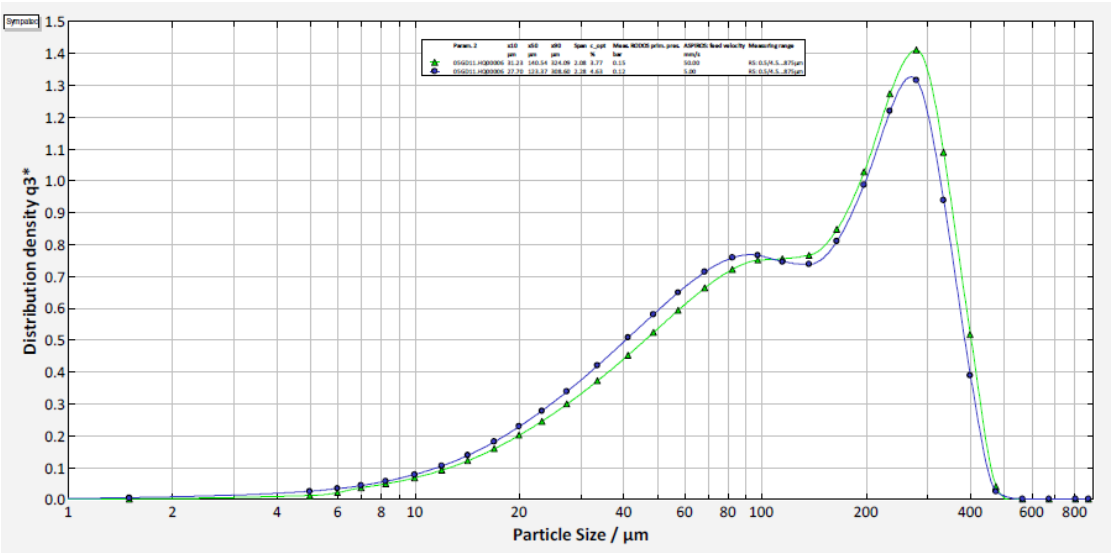
After choosing the lens, it is necessary to choose the pressure and the feeding rate velocity.

In Figure 3-17 and Figure 3-18 are represented four titration curves for pressure and feed rate velocity of [05GD11.HQ00006] batch.



. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
05GD11.HQ00006	19.27	92.93	272.33	2.72	3.47	1.50	50.00	R5: 0.5/4.5...875µm
05GD11.HQ00006	8.88	44.96	199.45	4.24	2.27	3.02	6.00	R5: 0.5/4.5...875µm
05GD11.HQ00006	18.09	87.88	281.84	3.00	3.09	1.01	6.00	R5: 0.5/4.5...875µm
05GD11.HQ00006	22.70	96.44	273.61	2.60	2.67	0.51	6.00	R5: 0.5/4.5...875µm

Figure 3-17 Pressure tritration curves of [05GD11.HQ0006]



Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
05GD11.HQ00006	31.23	140.54	324.09	2.08	3.77	0.15	50.00	R5: 0.5/4.5...875µm
05GD11.HQ00006	27.70	123.37	308.60	2.28	4.63	0.12	5.00	R5: 0.5/4.5...875µm

Figure 3-18 Feed rate velocity tritration curves of [05GD11.HQ0006]

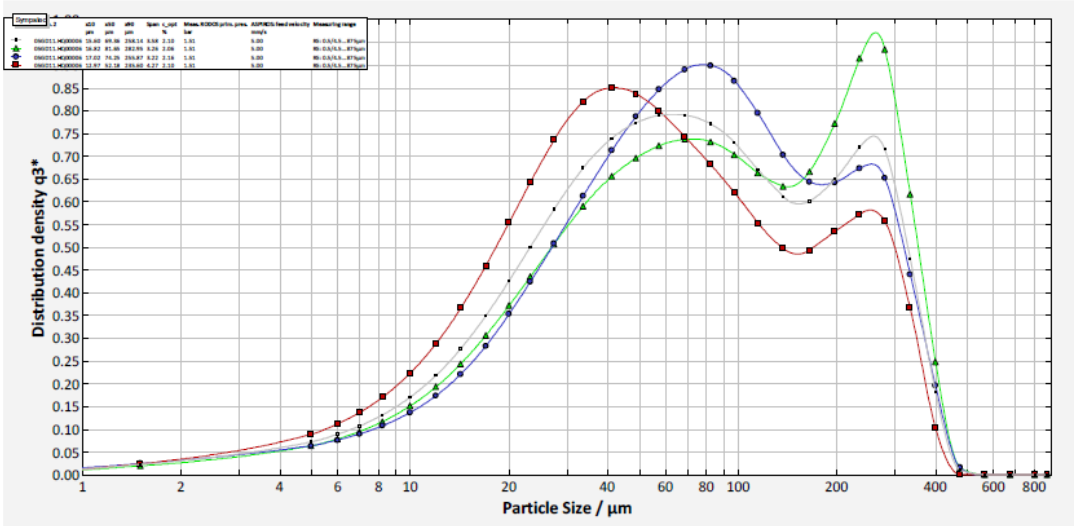
It is possible too see that there are two distinct particle size population. This result is concordant with the SEM images, where it can be seen a population of agglomerated and of agglomerated and scattered particles.

By observing the overlay of two curves in Figure 3-18 it is possible to see that the feed rate does not have a significant impact on the PSD.

At a pressure of 3 bar, it is observed a decrease in the PSD parameters indicating that the pressure employed for the analysis was too high.

The most promising curve was the one at 1.5 bar, which shown a well define curve and was in agreement with the SEM analysis information.

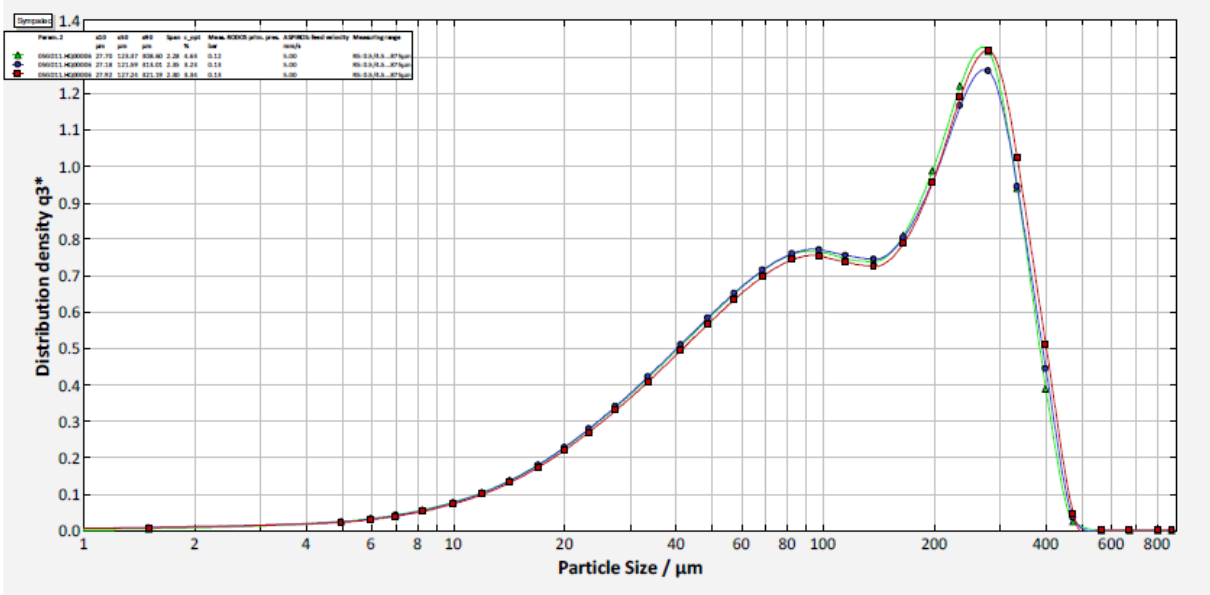
Since the product analyzed has a tendency to agglomerate, it was decided to lower the feeding rate speed and to increase the quantity of the sample in the vial. These alterations allowed the analysis of a more representative sample without promoting an overload in the detectors, since the feeding rate is low, a smaller quantity of particles is dispersed at a time.



Sympatec . 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
05GD11.HQ00006	15.60	69.36	258.14	3.58	2.10	1.51	5.00	R5: 0.5/4.5...875µm
05GD11.HQ00006	16.82	81.65	282.95	3.26	2.06	1.51	5.00	R5: 0.5/4.5...875µm
05GD11.HQ00006	17.02	74.25	255.87	3.22	2.16	1.51	5.00	R5: 0.5/4.5...875µm
05GD11.HQ00006	12.97	52.18	235.60	4.27	2.10	1.51	5.00	R5: 0.5/4.5...875µm

Figure 3-19 Repeatability curves of [05GD11.HQ0006] at a pressure of 1.5 bar and a feed rate velocity of 5 mm/s

The curves behavior in Figure 3-19 presented some variation so it was decided to decrease the pressure to 0.1 bar. This decision relates to the fact that there was some instability on the repeatability test, meaning that the pressure may be too high.



Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
05GD11.HQ00006	27.70	123.37	308.60	2.28	4.63	0.12	5.00	R5:0.5/4.5...875µm
05GD11.HQ00006	27.18	121.59	313.01	2.35	3.23	0.13	5.00	R5:0.5/4.5...875µm
05GD11.HQ00006	27.92	127.24	321.19	2.30	3.34	0.13	5.00	R5:0.5/4.5...875µm

Figure 3-20 Repeatability curves of [05GD11.HQ0006] at a pressure of 0.1 bar and a feed rate velocity of 5 mm/s

As it was expected the titration curves are similar between them, presented no signs of instability and the PSD values are in agreement with the SEM analysis.

In summary, the analytical conditions of the dry dispersion method for PSD analyzes of [GD11] unmicronized batches are described in Table 3-5.

Table 3-5 Analytical conditions of the dry dispersion method for PSD analyzes of [GD11]

Pressure (bar)	Feed velocity (mm/s)	Lens
0.1	5	R5

4. Results and discussion

The products analyzed in this chapter were processed by different techniques of particle engineering. The particle size was assessed using techniques of microscopy (SEM), and laser diffraction with liquid and dry dispersion techniques such as Malvern and Sympatec, respectively.

For product identification, Hovione has an internal code that is briefly explained in Figure 4-1.

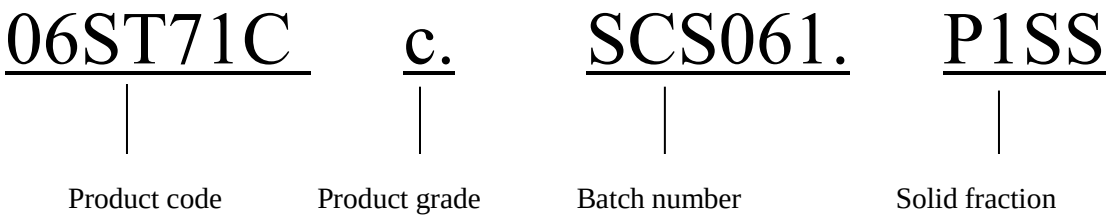


Figure 4-1 Example of product identification accordingly to Hovione's internal code

In this chapter, the results obtained during the course of this thesis are presented and discussed.

4.1 Particle Engineering by Wet Polishing

Wet polishing is a Hovione's particle engineering technology developed to generate 100% stable and crystalline micronized product that is subsequently isolated in a spray drying step [36].

Throughout this chapter, only results of dry fractions are presented. The wet fractions were analyzed while in suspension to closely monitor the PSD evolution during the WP process, before proceeding to the spray drying isolation step.

4.1.1 IH11c

[IH11] is the Hovione's internal code of an API to be used in a dry powder inhaler. This API was micronized with the goal of preparing micronized product with different PSD.

The target PS values for [IH11c] are described in Table 4-1.

Table 4-1 PSD target value for d(0.5) for [IH11c].

Batch 06IH11c.HQ00010	Batch 06IH11c.HQ00011
d(0.5)= 3.5 µm	d(0.5)= 3.5 µm
d(0.5)= 2.5 µm	d(0.5)= 2.5 µm
d(0.5)= 2 µm	d(0.5)= 1.5 µm
d(0.5)= 1.5 µm	

4.1.1.1 Batch 06IH11c.HQ00010

Before beginning the wet polishing process, the SRM was analyzed by SEM and the result is shown in Figure 4-2.

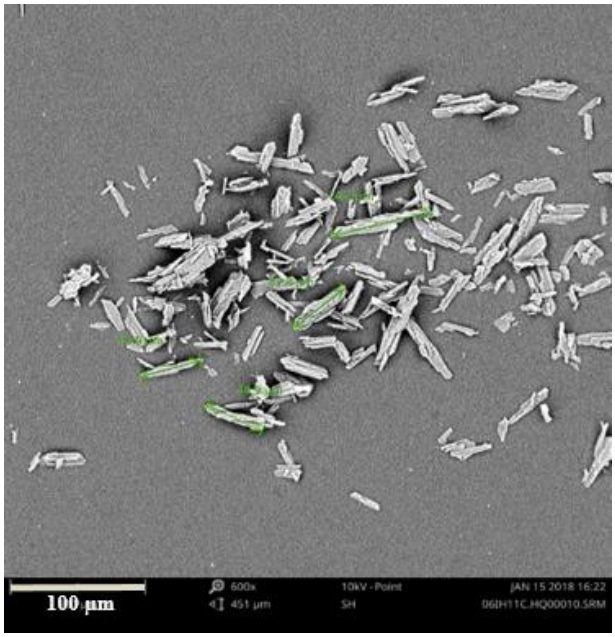


Figure 4-2 Powder sample of unmicronized material at a magnification of 600x -10kV -Point

By analyzing Figure 4-2, it can be observed that the particles exhibit a similar morphology and appear to have a size between 30 and 100 µm.

In order to reach the desirable PSD target, the unmicronized API went through several cycles of micronization using a specific milling camera.

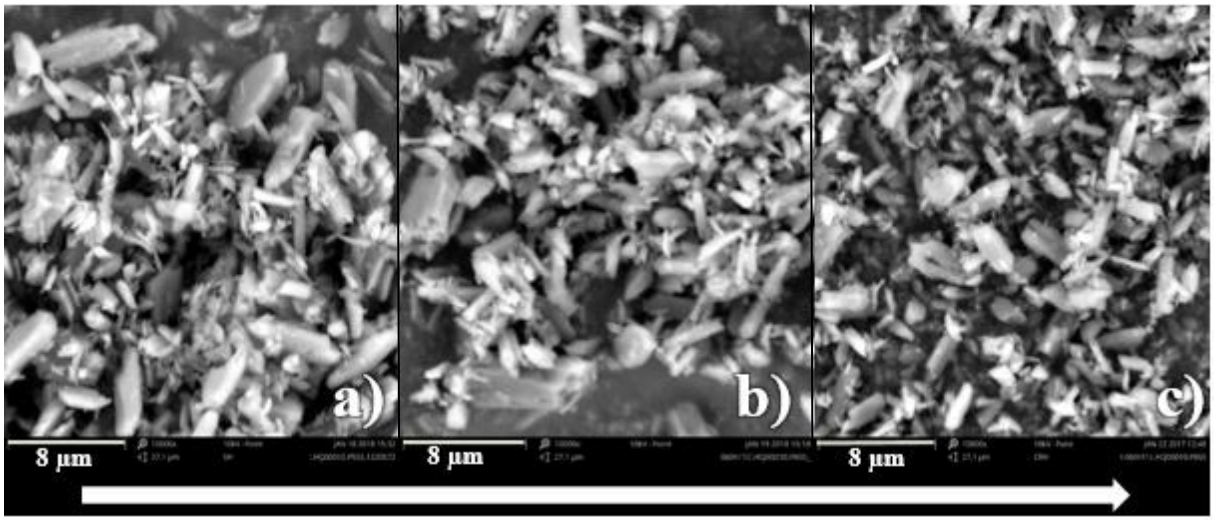


Figure 4-3 [06IH11c.HQ00010] micronization evolution. a) Product after micronization at a magnification of 8000x [06IH11c.HQ00010.P5SS]; b) Product after micronization at a magnification of 8000x [06IH11c.HQ00010.P6SS]; c) Product after micronization at a magnification of 8000x [06IH11c.HQ00010.P8SS].

Figure 4-3 shows the API shape and size as the micronization evolves: a) dry powder product after 10 cycles of micronization to reach a d (0.5) of 3.5 µm, b) after 15 cycles of micronization to reach a d (0.5) of 2.5 µm and c) after 25 cycles of micronization to reach a d (0.5) of 1.5 µm.

It is observed that the crystals particle size is gradually decreased as micronization evolves

4.1.1.1.1 Comparative analysis of SEM, Malvern and Sympatec

After analysing the particles shape and size by SEM, the PSD of the product was determined by means of laser diffraction techniques.

For the wet dispersion technique, the method used was available for PS analysis using MALVERN 2000 and validated by Hovione.

As described in the validated method, the dispersant used was acetone and sample preparation required the following steps: dry powder sample weighing, dispersant addition and vortex homogenization of the resulting suspension.

The optimal analytical conditions used are described in Table 4-2.

Table 4-2 Analytical conditions on wet dispersion for solid powders for product [IH11c]

Accessory	
Sample handling unit	Hydro 2000S
Stirrer speed (rpm)	2100
Ultrasonic amplitude (%) (dispersion unit US)	The amplitude is the one that will correspond to 66 +/- 5 V (as measured by Hydro US meter)
Time US (mins)	01:00
Measurement options	
Sample material name	IH11
Refractive index	1.52
Absorption	0.01
Dispersant name1)	Acetone
Refractive index of the dispersing agent	1.36
Result calculation	
Model	General purpose
Calculation sensitivity	Normal sensitivity
Particle shape	Irregular
Measurement	
Sample measurement time	10 seconds (10000 snaps)
Background measurement time	10 seconds (10000 snaps)
Number of measurements	3
Delay	10 seconds
Average	Create average result
Obscuration limits	5 – 11%

The average result of the Malvern 2000 analysis for the [06IH11c.HQ00010.P5SS] sample is presented in Figure 4-4, as well as the PSD curve.

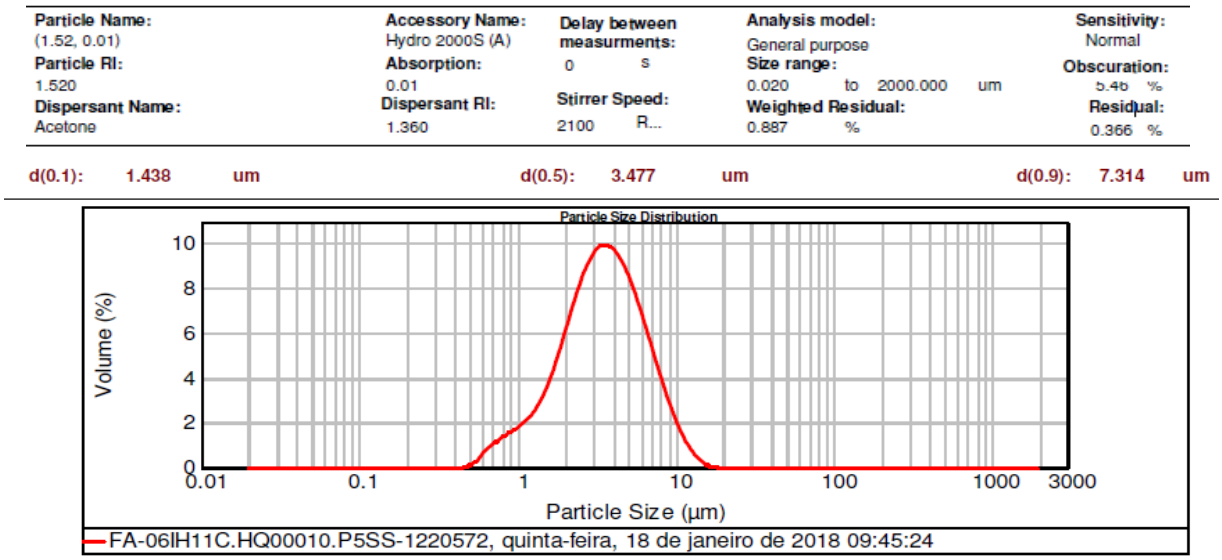


Figure 4-4 PSD result of the average value of the wet dispersion for [06IH11c.HQ00010.P5SS].

The lower residual value (lower than 2) and the fact that the obscuration value is within the range set defined in the method, validates de analysis.

The PSD curve represented in Figure 4-4 shows no signs of turbulence during analysis and shows the presence of fine particles population (seen at the left size of the PSD curve), indicating the presence of particles with PS lower than 1 µm.

When comparing the d(0.1), d(0.5) and d(0.9) average values obtained on MALVERN with the SEM images at a magnification of 8000x, it can be observed that the results are in good agreement.

In order to improve the assessment and efficiency of the PS analysis by wet dispersion, it was decided to analyze the product in Malvern 3000 as well. The development of the method for this equipment is explained in detail in chapter 3.

The conditions for the product PS analysis for Malvern 3000 are presented in Table 3-4 Table 3-4 Final analytical conditions for Malvern 3000 analysis of [IH11c] in chapter 3.

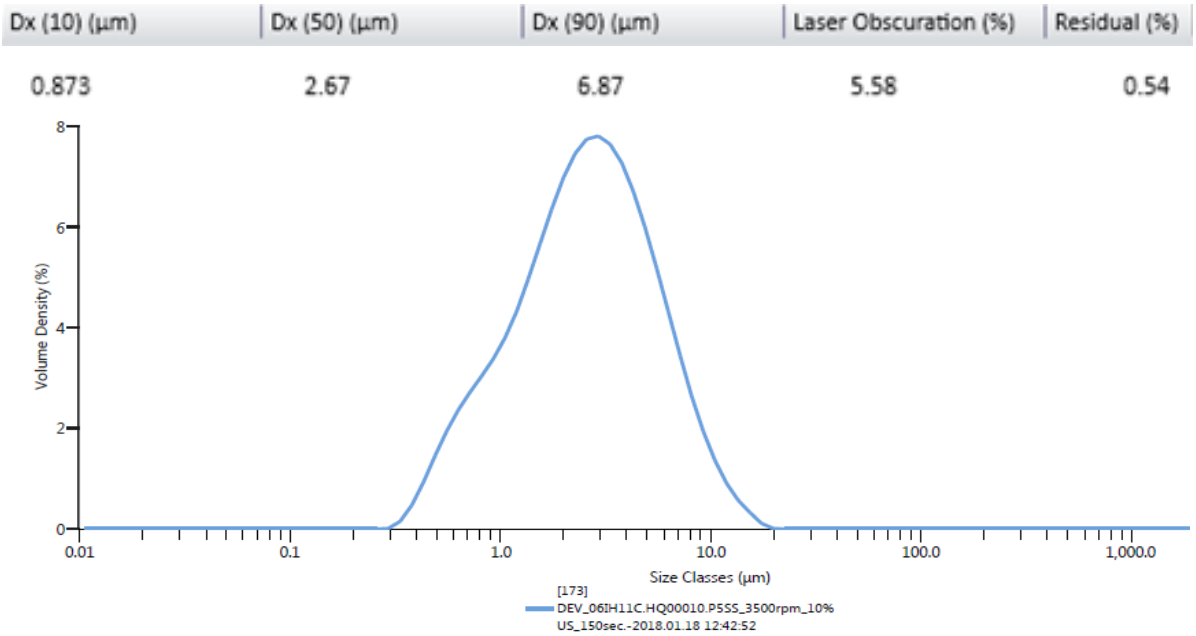


Figure 4-5 PSD curve of the average value by wet dispersion for [06IH11c.HQ00010.P5SS] using Malvern 3000.

As previously mentioned, the analysis is considered valid because the residual value is lower than 2 and the obscuration is within the range set in the method.

Generally, Malvern 3000 has a greater higher resolution than the Malvern 2000, which means that the fine particles population are measured more accurately. The PSD curve is in agreement with this fact as it shows a shift to the left, when compared with the PSD curve obtained by Malvern 2000. Consequently, the values of d(0.1), d(0.5) and d(0.9) obtained by Malvern 3000 are lower than the values obtained by Malvern 2000. However, the difference observed is less than one micron in the d(0.5), being the SEM analysis in agreement with both results.

Table 4-3 PSD results obtained from wet dispersion : Malvern 2000 vs Malvern 3000 analysis for [06IH11c.HQ00010.P5SS]

	d(0.1) µm	**d(0.5) µm**	**d(0.9) µm**
Malvern 2000	1.4	3.5	7.3
Malvern 3000	0.9	2.7	6.9
Difference (Mv 2000-Mv 3000)	0.6	0.9	0.6

For the dry dispersion, on Sympatec, the analytical method development follow the steps described in chapter 3. The pressure and feed rate velocity titration curves of the micronized product are shown in Appendix 7.III.

After analyzing the SEM images, it was concluded that the ideal conditions for the analysis were considered 0.5 bar at feed rate of 18 mm/s, using the R1 lens.

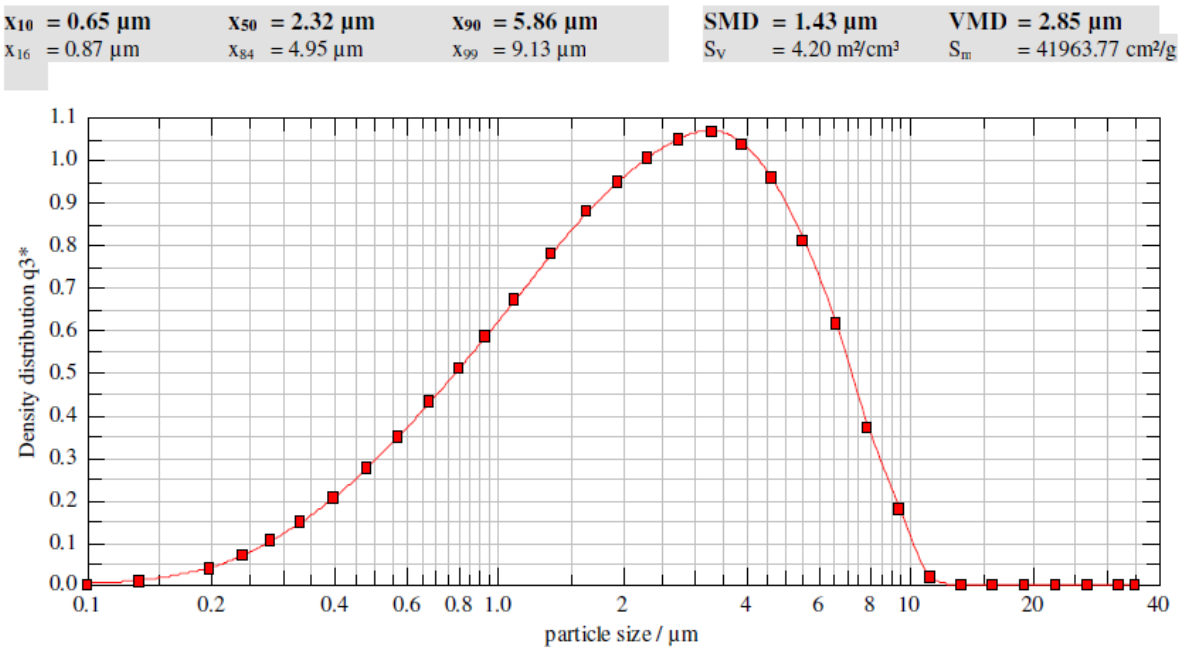


Figure 4-6 PSD curve on dry dispersion [06IH11c.HQ00010.P5SS]

Since the Malvern 3000 results were considered more accurate for the PS analysis, the comparison between dry and the wet dispersion was done considering Sympatec and Malvern 3000 results.

Table 4-4 PSD results obtained from Malvern 3000 vs Sympatecanalysis for [06IH11c.HQ00010.P5SS]

	d(0.1) µm	**d(0.5) µm**	**d(0.9) µm**
Malvern 3000	0.9	2.7	6.9
Sympatec	0.7	2.3	5.9
Difference (Mv - Spt)	0.2	0.4	1.0

By the PSD comparison, it can be observed that in boths methods, the d(0.1) and d(0.5) and d(0.9) values are in good agreement

For this product specifications, the critical attribute parameter was the d(0.5) value which is identical in both methods.

Two more dry powder samples were analyzed, with d(0.5) target value of 2.5 µm [06IH11c.HQ00010.P6SS] and of 1.5µm [06IH11.HQ00010.P8SS].

Table 4-5 and Table 4-6 summarize the results obtained by the different dispersion methods.

Table 4-5 PSD results obtained from wet dispersion and dry dispersion analysis from [06IH11c.HQ00010.P6SS] and [06IH11c.HQ00010.P8SS]

[06IH11c.HQ00010.P6SS]			
	d(0.1) µm	**d(0.5) µm**	**d(0.9) µm**
Malvern 2000	1.0	2.7	5.7
Malvern 3000	0.7	2.2	5.0
Sympatec(1 bar, 18 mm/s,R1)	0.6	2.0	4.5
[06IH11.HQ00010.P8SS]			
	d(0.1) µm	**d(0.5) µm**	**d(0.9) µm**
Malvern 2000	0.7	2.0	4.6
Malvern 3000	0.6	1.6	3.7
Sympatec(3 bar, 18 mm/s,R1)	0.6	1.4	3.3

Table 4-6 PSD results obtained from Malvern 3000 vs Sympatec analysis for [06IH11c.HQ00010.P6SS] and [06IH11c.HQ00010.P8SS]

[06IH11c.HQ00010.P6SS]			
	d(0.1) µm	**d(0.5) µm**	**d(0.9) µm**
Malvern 3000	0.7	2.2	5.0
Sympatec	0.6	2.0	4.5
Difference (Mv-Spt)	0.1	0.2	0.5
[06IH11c.HQ00010.P8SS]			
	d(0.1) µm	**d(0.5) µm**	**d(0.9) µm**
Malvern 3000	0.6	1.6	3.7
Sympatec	0.6	1.4	3.3
Difference (Mv-Spt)	0.0	0.2	0.4

By analyzing the PSD results of the last two fractions (P6SS and P8SS), it can be observed that in both methods, the d(0.1) and d(0.5) values are very similar. The d(0.9) shows a higher difference, however this difference shows a decrease as the product is further micronized .

The PSD targets were achieved and it can be stated that SEM, Malvern 3000 and Sympatec are in good agreement.

4.1.1.2 Batch 06IH11c.HQ00011

In this batch, the wet polishing and PS analysis were carried out in a very similar manner to the previous batch. In this batch it was used a different source of unmicronized product, what can be confirmed by in Figure 4-7 that show unmicronized product at two different magnifications (265x and 580x).

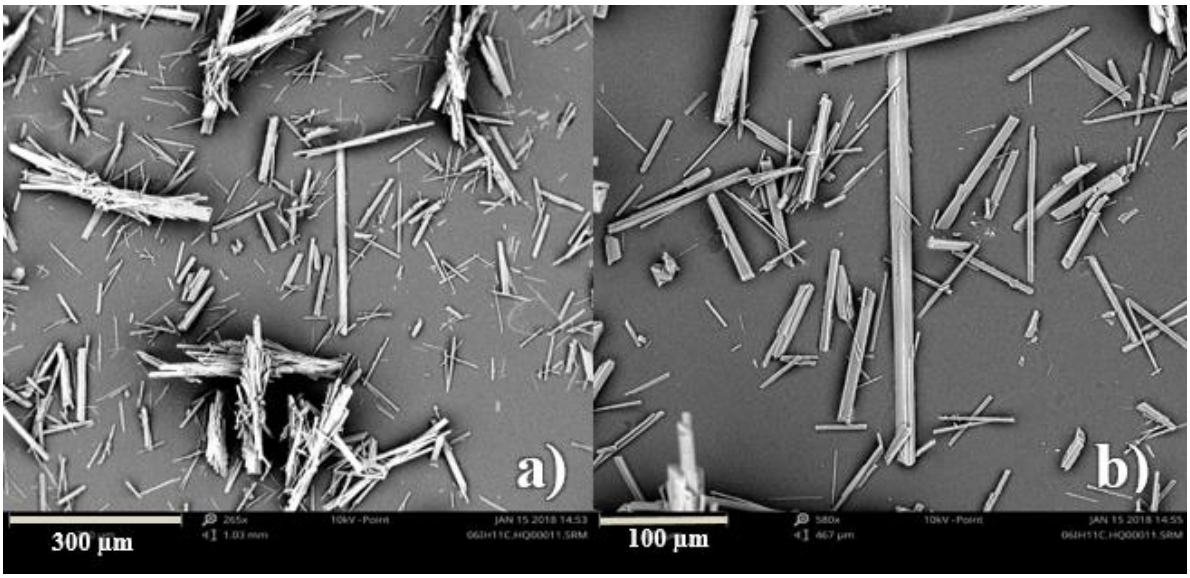


Figure 4-7 a) Unmicronized API at a magnification of 265x-10kV –Point, b) Unmicronized API at amagnification of 580x-10kV -Point

The SRM particles show a different morphology and size when compared to the SRM previous batch [06IH11c.HQ00010]. These particles are bigger and exhibit a needle-like shape.

Size wise, SEM shows that the bigger particles range from 100 to 350 µm.

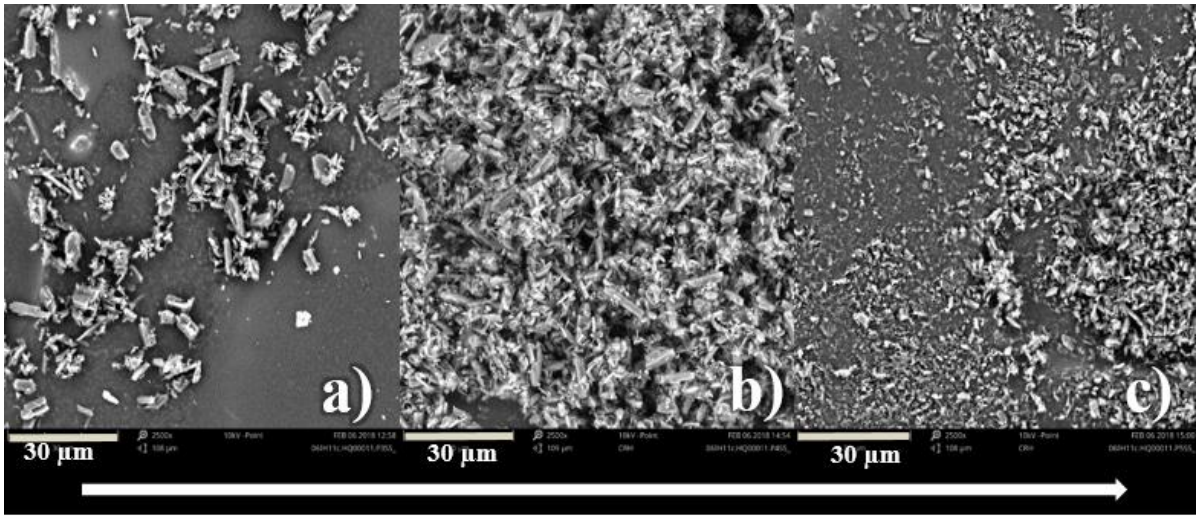


Figure 4-8 a) Product after micronization at a magnification 2500x [06IH11c.HQ00011.P3SS]; b) Product after micronization at a magnification 2500x[06IH11c.HQ00011.P4SS]; c) Product after micronization at a magnification 2500x [06IH11c.HQ00011.P5SS]

The SEM images in Figure 4-8 shows the API shape and size reduction during the micronization course of [06IH11c.HQ00011], where a) is a dry powder sample after 5 cycles of micronization to reach a d(0.5) of 3.5 µm, b) after 7 cycles of micronization to reach a d(0.5) of 2.5 µm and c) after 20 cycles of micronization to reach a d(0.5) of 1.5 µm.

Because of the size differences observed in the starting raw material of batch [HQ00011] when compared to the SRM of batch [HQ00010], the micronization process was adjusted so that the desired PSD target was attained. To obtain the last d(0.5) target value of 1.5 µm it was necessary process the product in a micronization chamber with a lower inner diameter.

4.1.1.2.1 Comparative analysis of SEM, Malvern and Sympatec

It was concluded by the analysis comparison of Malvern 2000 and Malvern 3000 performed for batch [06IH11c.HQ00010] that the method to be used in PS analysis of [IH11] products in wet dispersion was the method developed for Malvern 3000.

The optimal analytical conditions used are described in Table 3-4.

Analysis		Result	
Particle Name	IH11	**Concentration**	0.0013 %
Particle Refractive Index	1.650	**Span**	2.646
Particle Absorption Index	1.000	**Uniformity**	0.859
Particle Refractive Index Blue	1.650	**Specific Surface Area**	3094 m^2/kg
Particle Absorption Index Blue	1.000	**D [3;2]**	1.85 µm
Dispersant Name	Acetone	**D [4;3]**	3.76 µm
Dispersant Refractive Index	1.360	**Dv (10)**	0.813 µm
Analysis Model	General Purpose	**Dv (50)**	2.67 µm
Single Result Mode?	No	**Dv (90)**	7.87 µm
Weighted Residual	0.64 %	**Mode**	2.82 µm
Laser Obscuration	5.82 %	**Result Units**	Volume
Residual	0.59 %	**Sample Measurement Time**	10.00 s
Stirrer Speed Achieved	3500 rpm	**Laser Power**	79.84 %
Accessory Name	Hydro MV		
Analysis Sensitivity	Normal		
Particle Density	1.05 g/cm^3		

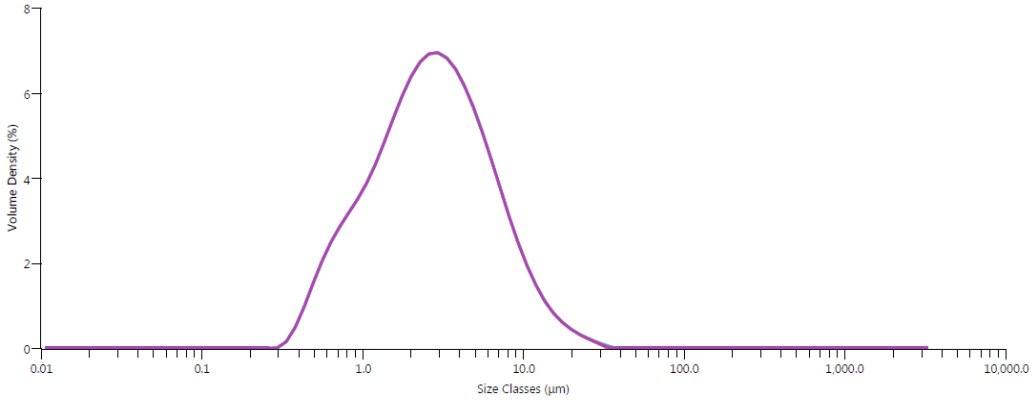


Figure 4-9 Result of the average PSD values obtained by wet dispersion for [06IH11c.HQ00011.P4SS] in Malvern 3000.

The lower residual value and the fact that the obscuration is within the range set validates de analysis.

The PSD curve represented in the Figure 4-9 confirms the absence of turbulence during analysis. The d(0.1), d(0.5) and d(0.9) average values obtained in Malvern are supported by with the SEM image (Figure 4-8 b)).

For the dry dispersion laser diffraction method developed in Sympatec, the ideal conditions determined for the analysis were 2 bar at a feed rate of 18 mm/s using the R1 lens.

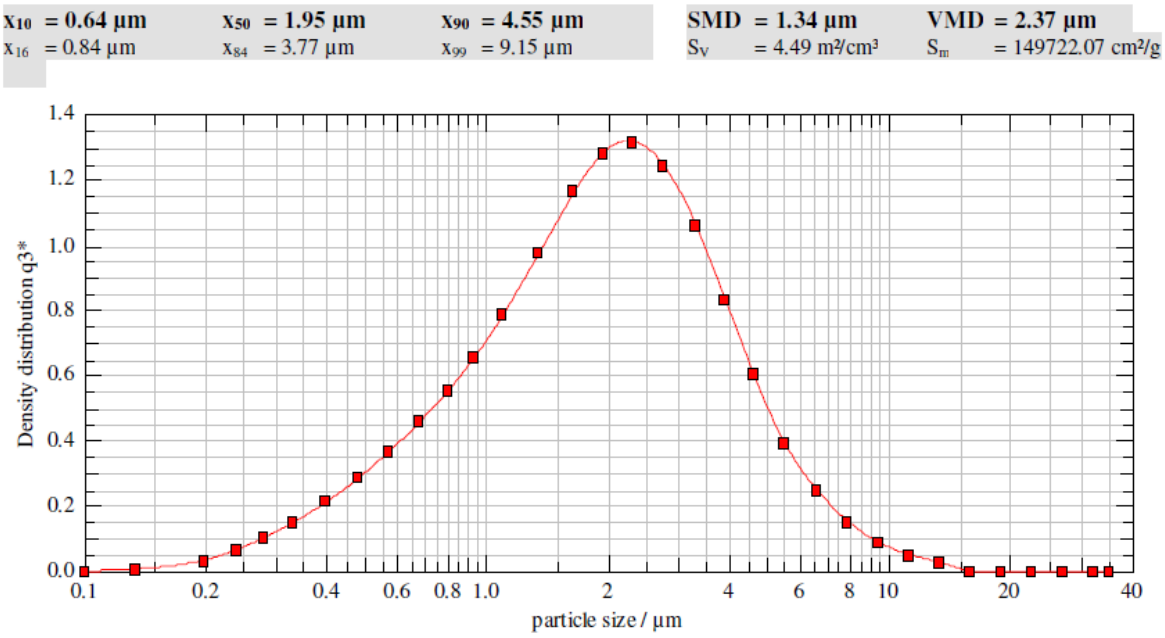


Figure 4-10 PSD results in dry dispersion (Sympatec) for [06IH11c.HQ00011.P4SS], using R1 lens, feed rate of 18mm/s and a pressure of 2 bar.

In Table 4-7 are summarized the PSD results obtained using two methods of laser diffraction

Table 4-7 PSD results obtained from Malvern 3000 vs Sympatec analysis for [06IH11c.HQ00011.P4SS]

	d(0.1) µm	**d(0.5) µm**	**d(0.9) µm**
Malvern 3000	0.8	2.7	7.9
Sympatec	0.6	1.9	4.6
Difference (Mv3000-Spt)	0.2	0.8	3.3

By comparing the PSD of the two methods, it can be observed that in both methods, the d(0.1) value is similar. However de value of d(0.9) and d(0.5) are higher in M3000 than in Sympatec.

Two more dry powder samples were analyzed, with d(0.5) target value of respectively 2.5 µm [06IH11c.HQ00011.P3SS] and 1.5µm [06IH11.HQ00011.P5SS].

Table 4-8 PSD results obtained by Malvern 3000 and Sympatecanalysis for [06IH11c.HQ00011.P3SS] and [06IH11c.HQ00011.P5SS]

[06IH11c.HQ00011.P3SS]			
	d(0.1) µm	**d(0.5) µm**	**d(0.9) µm**
Malvern 3000	0.9	3.4	10.5
Sympatec(1.5 bar,6 mm/s, R1)	0.7	2.7	7.9
Difference(Mv300-Spt)	0.2	0.7	2.6
[06IH11c.HQ00011.P5SS]			
	d(0.1) µm	**d(0.5) µm**	**d(0.9) µm**
Malvern 3000	0.6	1.5	3.8
Sympatec(3 bar, 6 mm/s, R1)	0.5	1.4	3.5
Difference(Mv300-Spt)	0.1	0.1	0.3

The fractions analyzed by both methods are in good agreement as little differences are observed the three PSD parameters. As the PS of the product is reduced, it is observed a higher similarity between the results obtained by both techniques.

4.1.2 ST71c

[ST71c] it is the Hovione's internal code for a steroid API. This product was micronized with the goal of prepare micronized product with specific PSD: d(0.5)= 2.9 µm and d(0.9)=6 µm.

4.1.2.1 Batch 06ST71c.SCS061

Prior the start of wet polishing, SEM images of the SRM were collected and are shown in Figure 4-11.

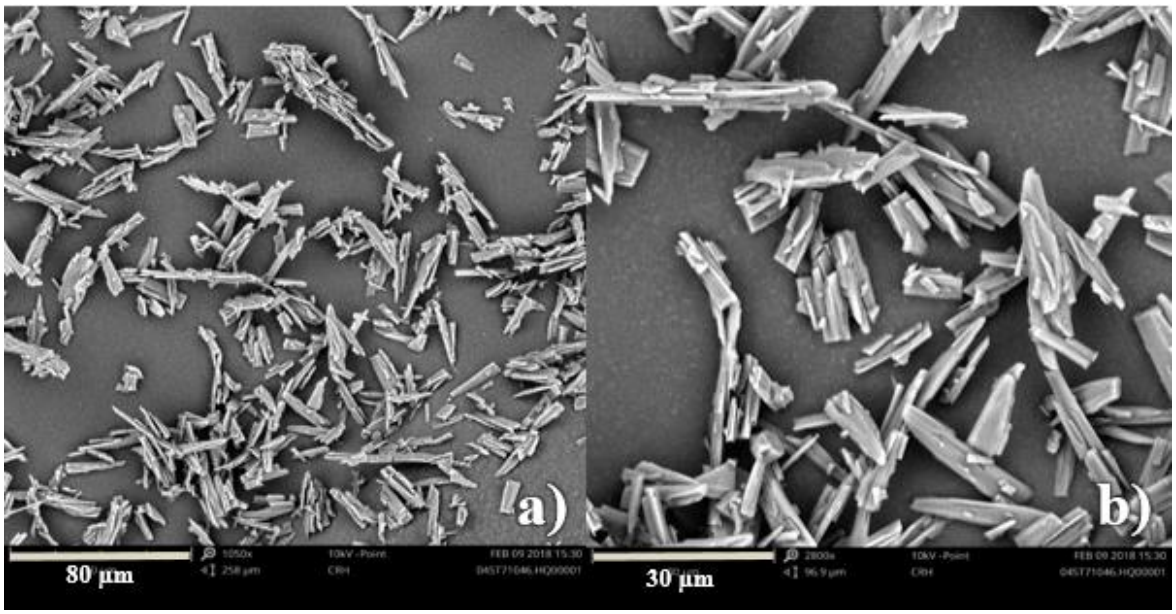


Figure 4-11 a) Powder sample of unmicronized material at a magnification of 1050x-10kV –Point; b) Powder sample of unmicronized material at a magnification of 2800x-10kV –Point

In Figure 4-11, it is possible to see that the SRM present particles with an irregular and rectangular shape. As can be seen, the particles of the SRM range from 2 µm to 55 µm, although some bigger agglomerates of approximately 50 µm to 80 µm can be observed.

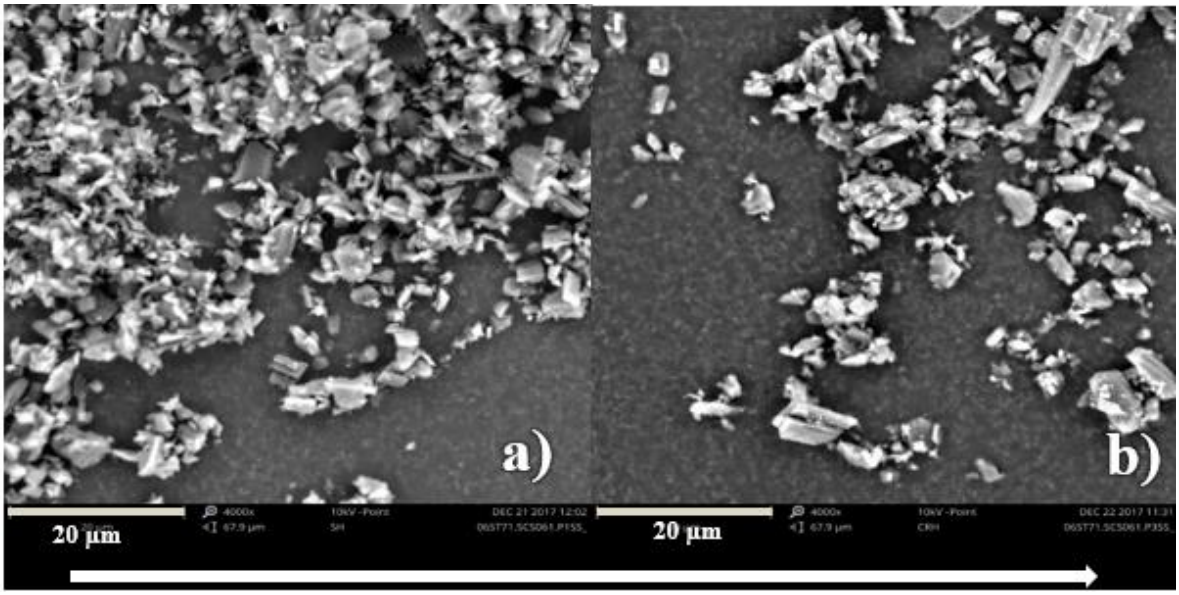


Figure 4-12 [06ST71c.SCS061] micronization evolution (particle size gradual decreased) a) Product after micronization at a magnification 4000x [06ST71c.SCS061.P1SS]; b) Product after micronization at a magnification 4000x [06ST71c.SCS061.P3SS].

After the wet micronization process, the particles are clearly smaller but still exhibit irregular outline with a particle size ranging between 3-8 µm.

4.1.2.1.1 Comparative analysis of SEM, Malvern and Sympatec

The particle size of the product was assessed by Malvern and Sympatec. Hovione has an analytical method validated available for the determination of the PS using Malvern, which was used in this study. The optimal analytical conditions used are described in Table 4-9.

Table 4-9 Analytical conditions for the wet dispersion method for solid powders of the product [ST71c]

Accessory	
Sample handling unit	Hydro 2000S
Stirrer speed (rpm)	2100 rpm
Ultrasonic (%)	50 %
Time of US	210 seconds
Measurement options	
Refractive index	1.52
Absorption	0.1
Dispersant name	Water
Refractive index of the dispersant agent	1.33
Result calculation	
Model	General Purpose
Calculation sensitivity	Normal sensitivity
Particle shape	Irregular
Measurement	
Sample measurement time	10 seconds (10000 snaps)
Background measurement time	10 seconds (10000 snaps)
Number of measurements	3
Delay	10 seconds
Average	Create average result
Obscuration limits	5 % - 9 %

The dispersant used was water with 0.1 mL of surfactant (10% (w/v) of SLS aq. Solution).

After the sample preparation and slurry was done according to the analytical validated method, the samples were analyzed.

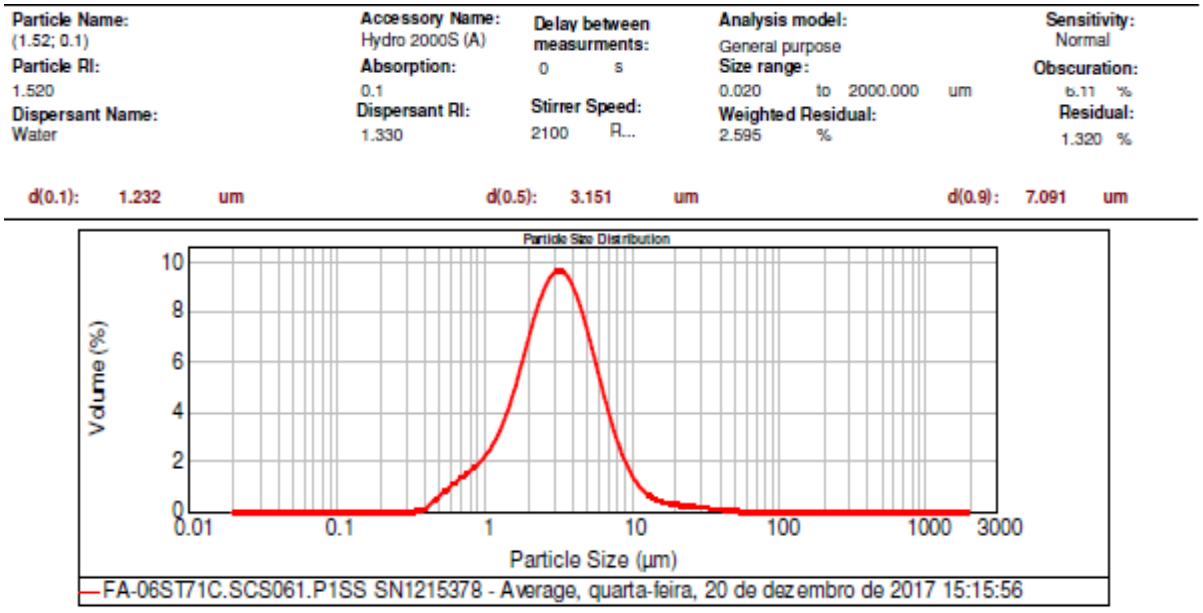


Figure 4-13 Result of the average PSD values of wet dispersion for [06ST71c.SCS061.P1SS].

Particle Name:	**Accessory Name:**	**Delay between measurments:**	**Analysis model:**	**Sensitivity:**
(1.52; 0.1)	Hydro 2000S (A)		General purpose	Normal
Particle RI:	**Absorption:**	0 s	**Size range:**	**Obscuration:**
1.520	0.1		0.020 to 2000.000 um	5.32 %
Dispersant Name:	**Dispersant RI:**	**Stirrer Speed:**	**Weighted Residual:**	**Residual:**
Water	1.330	2100 R...	2.773 %	1.540 %

d(0.1): 1.097 um **d(0.5):** 2.798 um **d(0.9):** 5.604 um

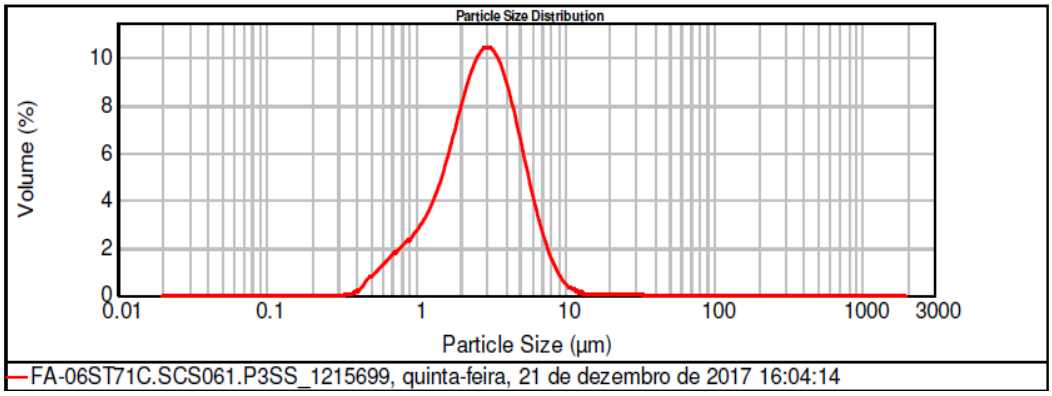


Figure 4-14 Result of the average PSD values of wet dispersion for [06ST71c.SCS061.P3SS].

The analysis wer considered valid and presented no signs of instability. The obscuration level was within the defined range and the residual value is under 2 % for both samples.

The PSD curves represented in Figure 4-13 and in Figure 4-14 denote the presence of fine population (on the left).

By comparing the dv (0.1), dv (0.5) and dv (0.9) average values obtained on Malvern for both samples with the SEM images at a magnification of 4000x, it can be observed that the results are in good agreement.

The dry dispersion method on Sympatec was developed according to the guidelines of the Sympatec development method described on chapter 3. The pressure and feed rate velocity titration curves of the micronized product are shown in Appendix 7.IV.

The ideal analytical conditions were considered 6 bar with a feeding rate18 mm/s using R1 lens.

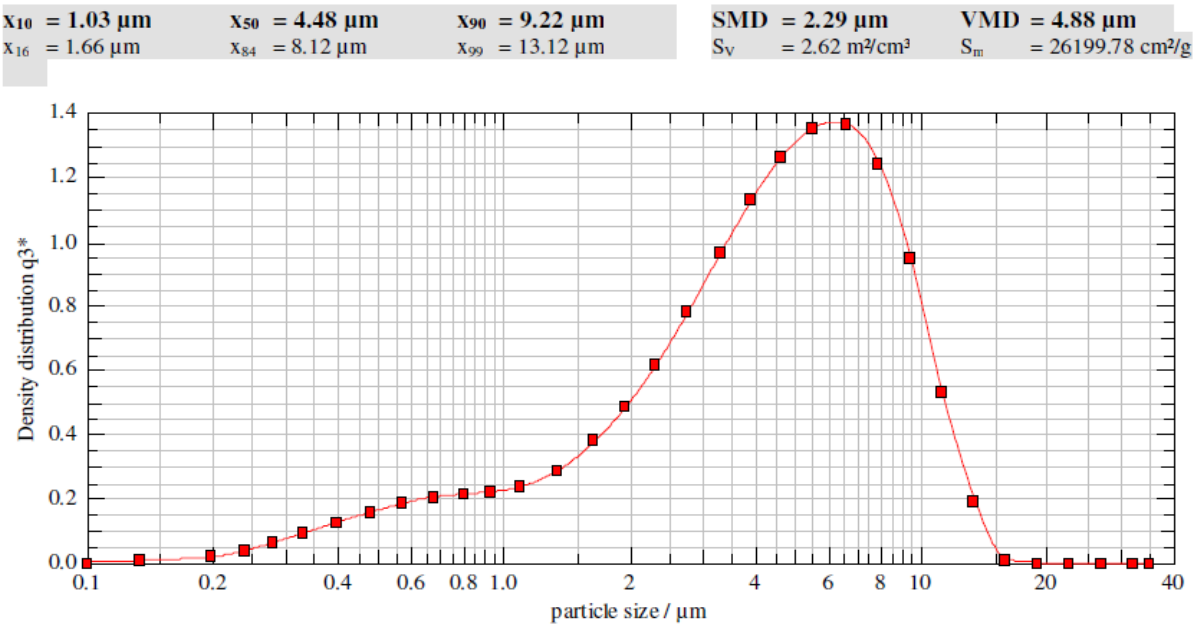


Figure 4-15 PSD on dry dispersion for [06ST71c.SCS061.P1SS]

The [06ST71c.SCS061.P3SS] fraction was obtained performing more micronization cycles. Consequently, the size of the particles obtained was lower and the ideal conditions were considered 6 bar at a feeding rate of 18 mm/s using the R1 lens.

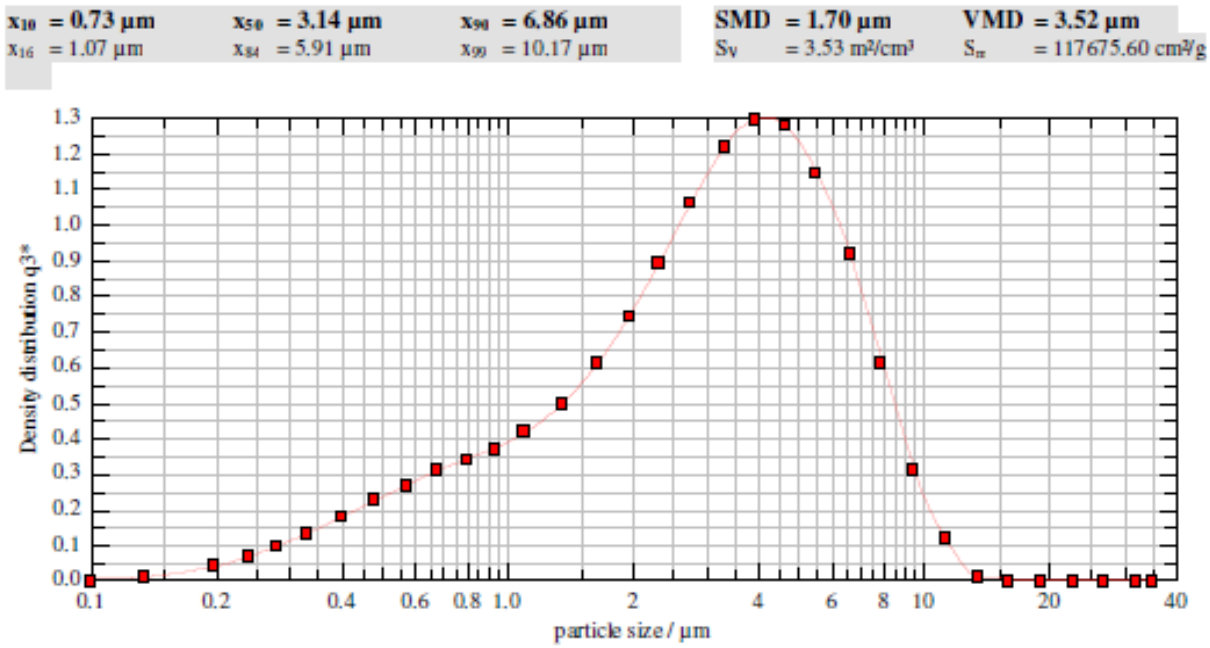


Figure 4-16 PSD on dry dispersion for [06ST71c.SCS061.P3SS]

Table 4-10 PSD results obtained from Malvern 2000 vs Sympatec analysis for [06ST71c.SCS061.P1SS] and [06ST71c.SCS061.P3SS]

[06ST71c.SCS061.P1SS]			
	d(0.1) µm	**d(0.5) µm**	**d(0.9) µm**
Malvern 2000	1.2	3.2	7.0
Sympatec(6 bar,18 mm/s, R1)	1	4.5	9.2
Difference (Mv2000-Spt)	0.2	-0.7	-2.2
[06ST71c.SCS061.P3SS]			
	d(0.1) µm	**d(0.5) µm**	**d(0.9) µm**
Malvern 2000	1.0	2.8	5.6
Sympatec(6 bar, 18 mm/s, R1)	0.7	3.1	6.9
Difference (Mv2000-Spt)	0.3	-0.3	-1.3

As can be noted, the fractions analyzed presented small differences regarding all PSD parameters.

Sympatec results are slightly higher than those obtained with Malvern, what can be partially explained by the different theoretical models used in the equipment.

4.1.3 ST52c

ST52c is a Hovione's internal code for steroid API. This product was micronized to obtain a specific PSD: 11µm < d(0.9) < 12µm, according to a client's request.

4.1.3.1 Batch 06ST52c.SCS058

The unmicronized crystals used as SRM was analyzed by SEM and the images are presented in Figure 4-17.

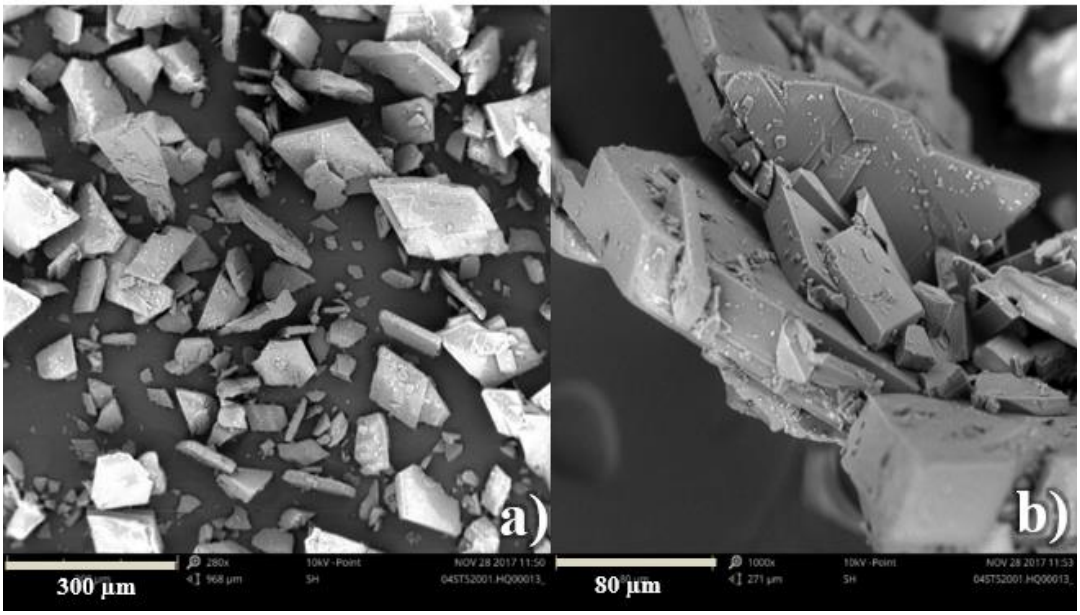


Figure 4-17 a) Powder sample of unmicronized material at a magnification of 1050x-10kV –Point; b) Powder sample of unmicronized material at a magnification of 2800x-10kV –Point

The unmicronized material appear to have smaller and larger particles with a laminar shape. The biggest particle observed was around 100 µm in diameter but the majority appeared to have a diameter between 50 and 90 µm.

Based on these images the wet polishing process was defined and pictures of the resulting dry fractions are presented in Figure 4-18.

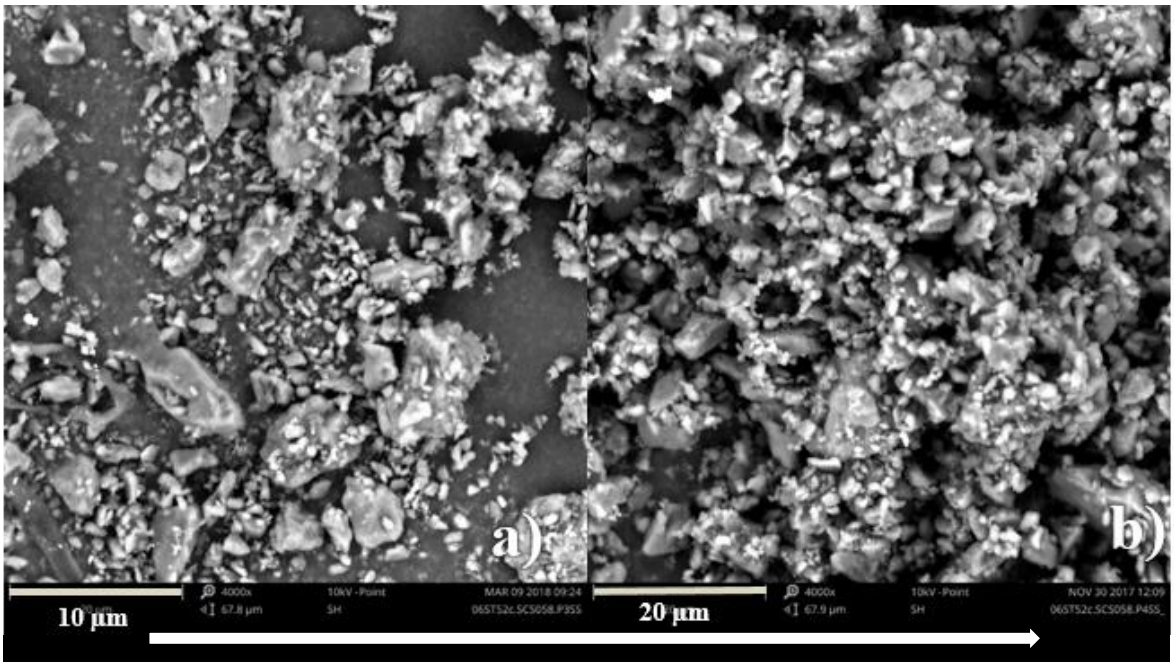


Figure 4-18 [06ST52c.SCS068] micronization evolution (particle size gradual decreased); a) Product after micronization at magnification of 4000x [06ST52c.SCS058.P3SS]; b) Product after micronization at a magnification of 4000x [06ST52c.SCS058.P4SS].

After the wet micronization process, it is possible to see a clear reduction in the particle size and change in the shape of the crystals. The smaller particles are approximately 1 µm in diameter while the larger particles visible range between 10 and 20µm.

4.1.3.1.1 Comparative analysis of SEM, Malvern and Sympatec

After SEM analysis, the PSD of the product was determined by laser diffraction techniques.

There was an analytical method validated by Hovione available for PSD analysis using Malvern 2000S.

The validated analytical conditions of the method are described in Table 4-11.

Table 4-11 Analytical conditions of the wet dispersion method (Malvern) for solid powders of product [ST52c]

Accessory	
Sample handling unit	Hydro 2000S
Stirrer speed (rpm)	2100
Ultrasonic (%)	50%
Measurement options	
Material	
Sample material name	ST52c
Refractive Index	1.52
Absorption	0.01
Dispersant name	Water
Refractive Index of the dispersing agent	1.33
Result calculation	
Model	General purpose
Calculation sensitivity	Normal sensitivity
Particle shape	Irregular
Measurement	
Sample measurement time	10 seconds (10000 snaps)
Background measurement time	10 seconds (10000 snaps)
Number of measurements	3
Delay	10 seconds
Average	Create average result
Obscuration limits	10 – 20%

For sample preparation is necessary to add the surfactant/dispersant solution 30% (v/v) Tween 80 in water and slurry the sample. This procedure must be carefully performed in order to achieve a good contact between the particles and surfactant/dispersant and to efficiently promote particle de-agglomeration. The dispersant used is this method was water.

The samples were analyzed immediately after preparation and the PSD results are presented in Figure 4-19 and in Figure 4-20.

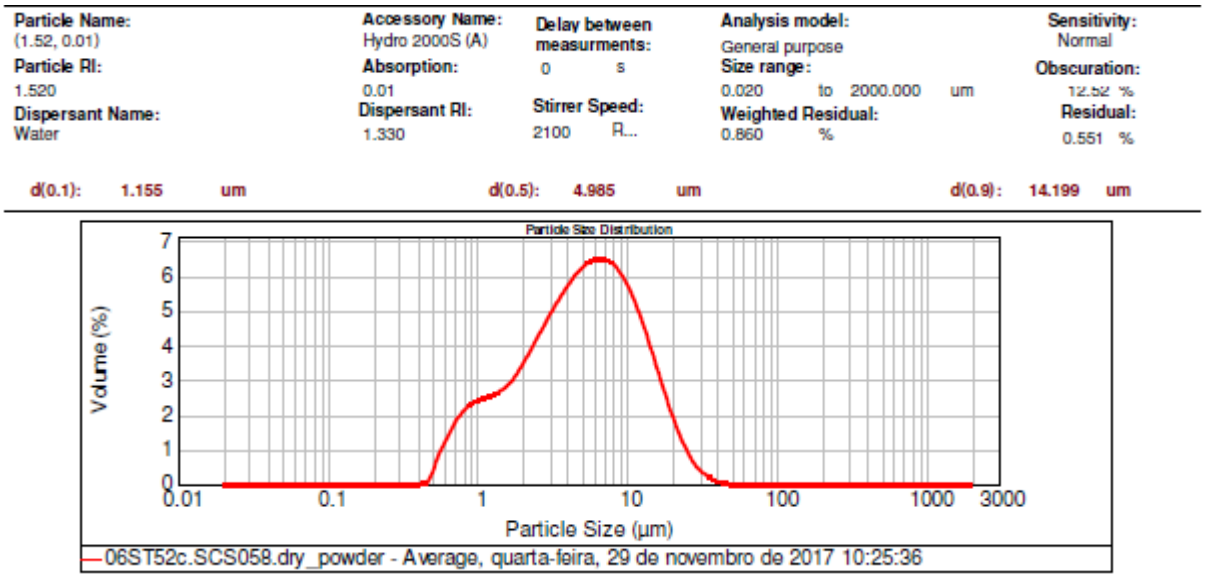


Figure 4-19 Result of the average PSD values of wet dispersion for [06ST52c.SCS058.P3SS].

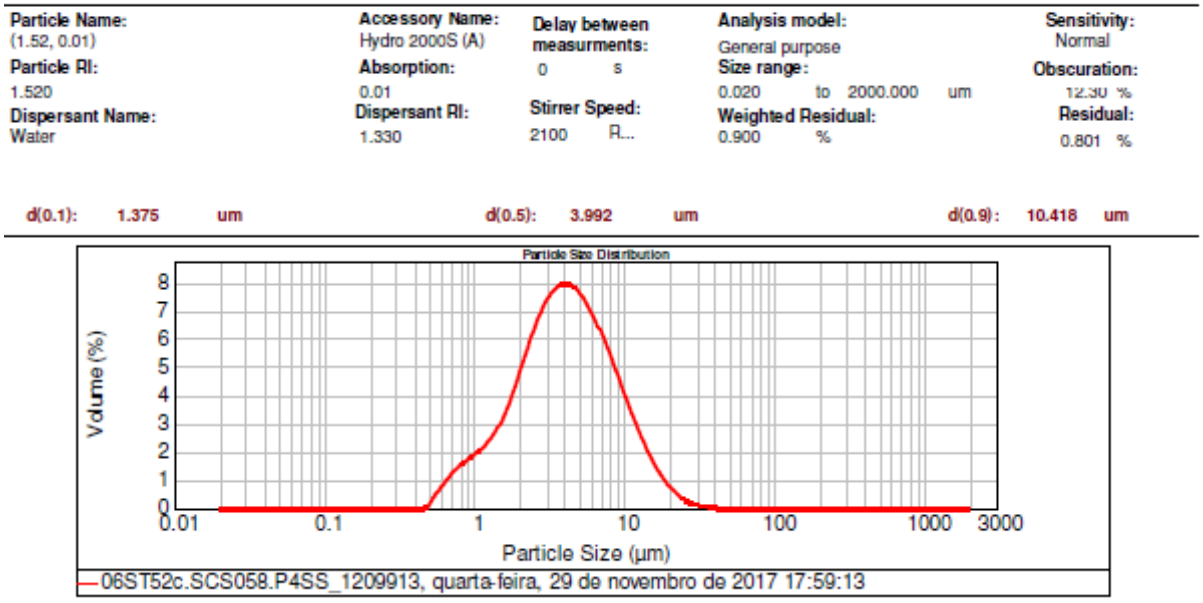


Figure 4-20 Result of the average PSD values of wet dispersion for [06ST52c.SCS058.P4SS].

The obscuration level is within the defined range and the residual value is under 2 % in both samples (Figure 4-19 and Figure 4-20) denote the presence of a fine population of fines.

The d(0.1), d (0.5) and d (0.9) average values determined for both fractions (P3SS and P4SS) on Malvern are in good agreement with the SEM images at a magnification of 4000x, since the bigger particles observed in SEM had a diameter of approximately 10 µm.

The dry dispersion method on Sympatec was developed as previously reported (chapter 2). The pressure and feed rate velocity titration curves of the micronized product are shown in Appendix 7.V.

The ideal conditions for the analysis in Sympatec were considered 5 bar at a feed rate of 50 mm/s using the R1 lens.

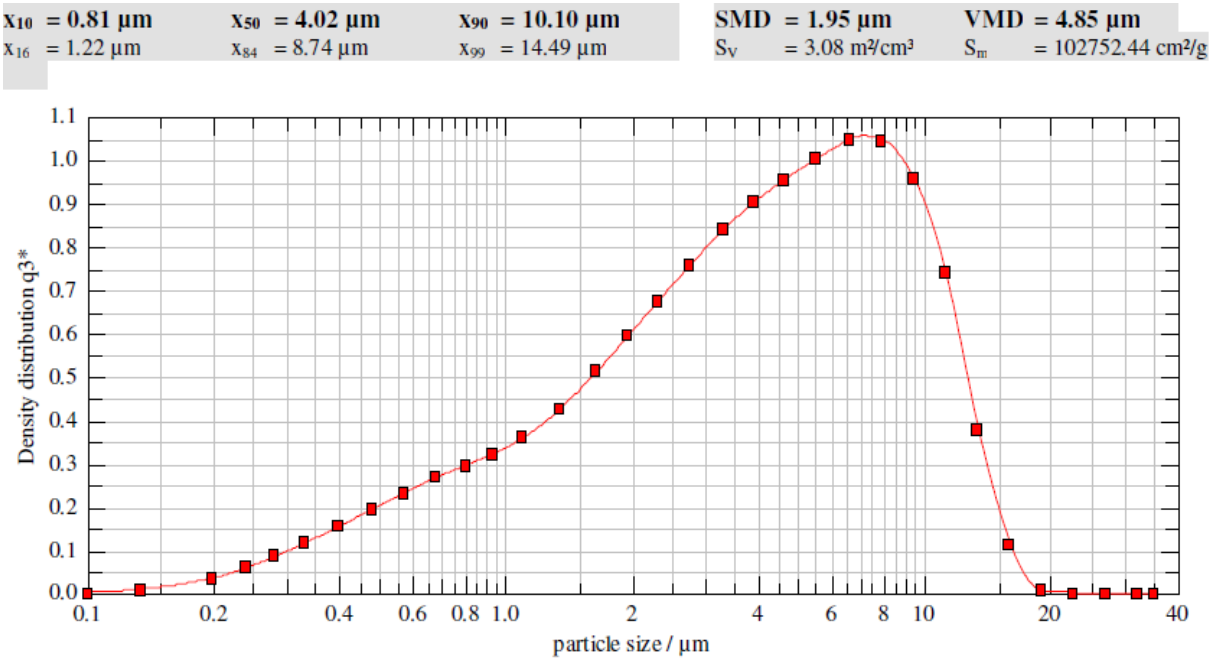


Figure 4-21 PSD of product [06ST52c.SCS058.P3SS] determined by Sympatec.

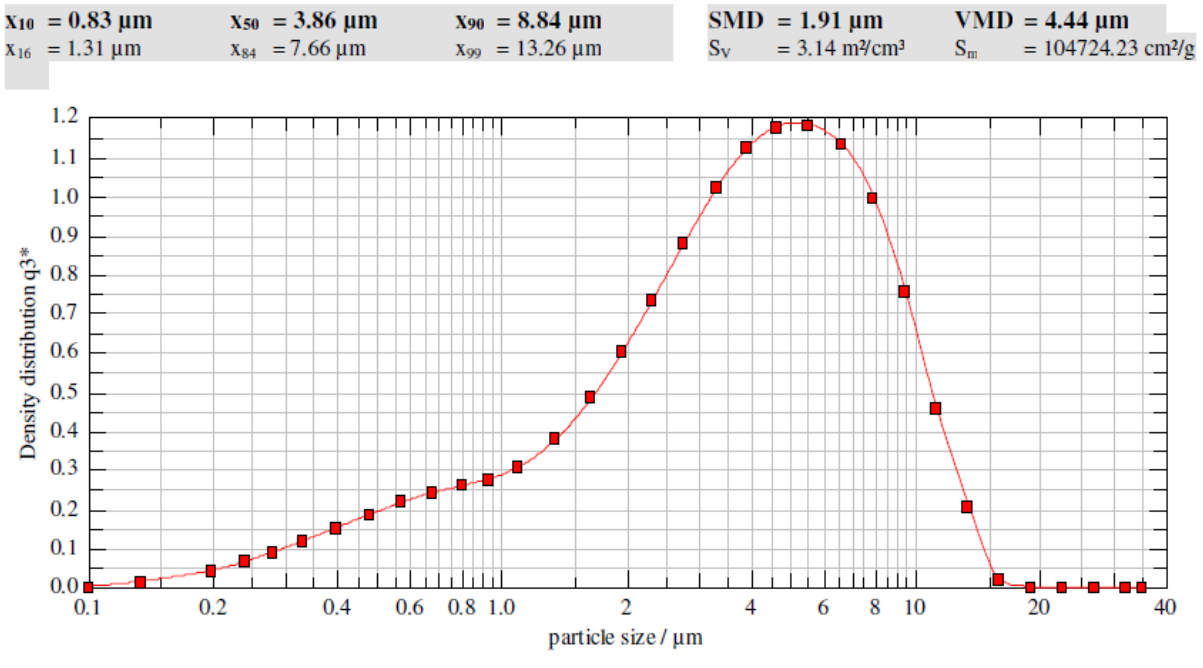


Figure 4-22 PSD of product [06ST52c.SCS058.P4SS] determined by Sympatec

Table 4-12 PSD results obtained from Malvern 2000 vs Sympatec for [06ST52c.SCS058.P3SS] and [06ST62c.SCS058.P4SS]

[06ST52c.SCS058.P3SS]			
	d(0.1) µm	**d(0.5) µm**	**d(0.9) µm**
Malvern 2000	1.2	5.0	14.2
Sympatec (5 bar,18 mm/s, R1)	0.8	4.0	10.1
Difference(Mv2000-Spt)	0.4	1.0	4.1
[06ST52c.SCS058.P4SS]			
	d(0.1) µm	**d(0.5) µm**	**d(0.9) µm**
Malvern 2000	1.4	4.0	10.4
Sympatec (6 bar, 18 mm/s, R1)	0.8	3.9	8.8
Difference (Mv2000-Spt)	0.6	0.1	1.6

Analyzing Table 4-12 it is possible to see that the fractions analyzed presented small differences regarding all PSD parameters what can be partially explained by the different theoretical models used by the two laser diffraction techniques.

4.2 Particle Engineering by Spray Drying

Spray drying is a continuous process that can be used for multiple applications. Over the last ten years, the technology has been used for the production of solid dispersions and is the fastest growing platform to overcome the solubility issues of oral drugs, being also relevant to inhalable particles, especially excipients and for the isolation of thermally labile products [17].

4.2.1. KB21S

KB21S is the Hovione's internal code for API intended for use in ophthalmic delivery. A specific form of this API was prepared by spray drying aiming at PSD target of Dv90< 4 µm.

4.2.1.1 Batch 06KB21S.SCS059

The morphology and size of the starting raw material before spray drying process is shown in Figure 4-23.

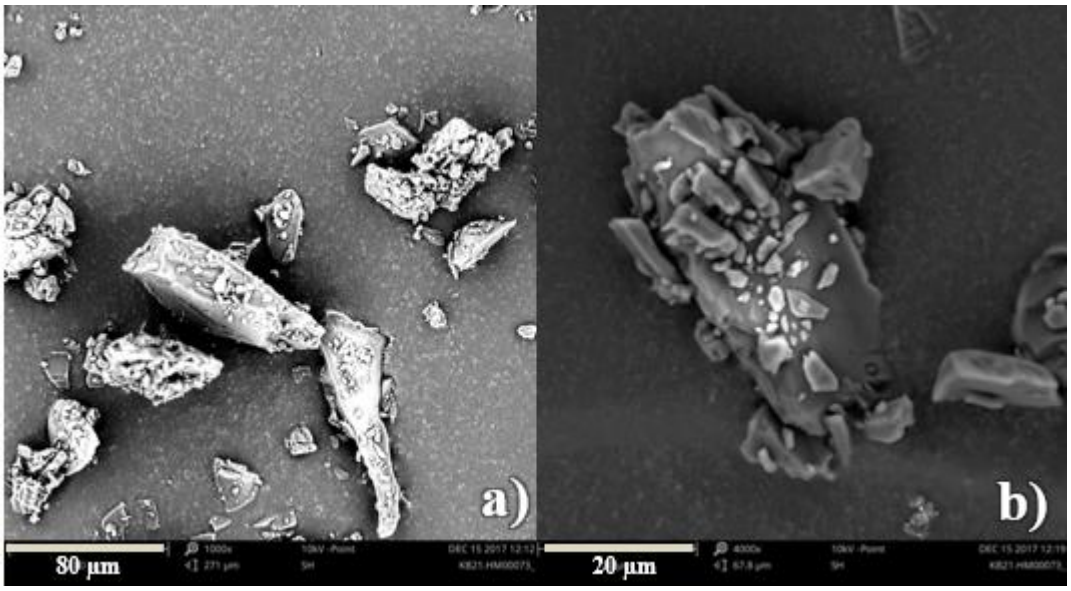
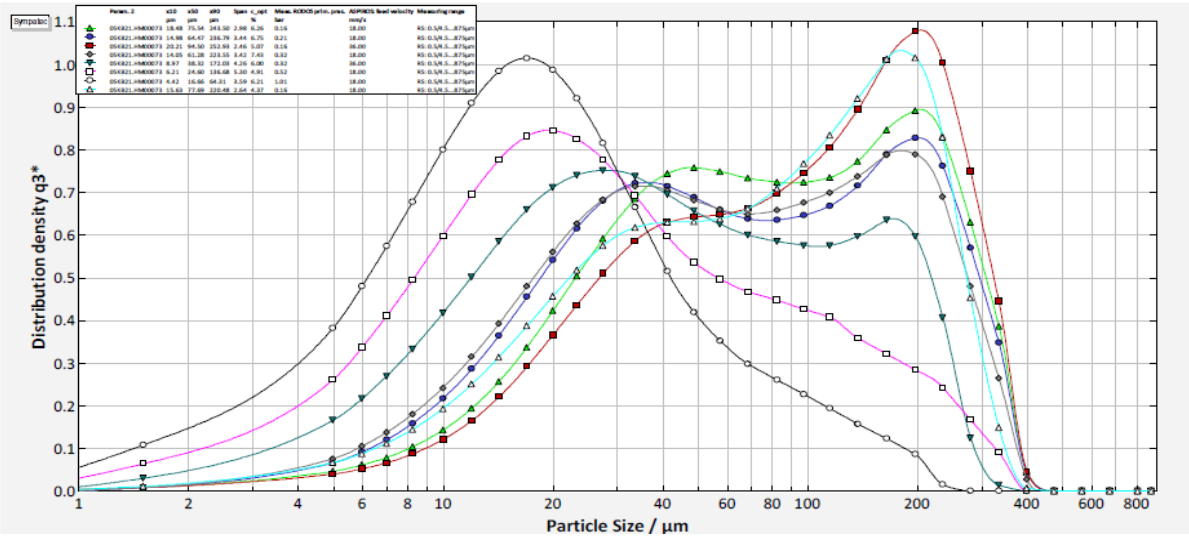


Figure 4-23 a) Powder sample of KB21S at a magnification of 1000x-10kV and b) at a magnification of 4000x-10kV

The SRM particle size assessment was carried out using Sympatec because there was no wet dispersion method developed and validated by Hovione. A Sympatec PS method was established according to the guidelines of the Sympatec development method described in detail in chapter 3. The pressure and feed rate velocity titration curves of the SRM are shown in Figure 4-24.



Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
05KB21.HM00073	18.48	75.54	243.50	2.98	6.26	0.16	18.00	R5: 0.5/4.5...875µm
05KB21.HM00073	14.98	64.47	236.79	3.44	6.75	0.21	18.00	R5: 0.5/4.5...875µm
05KB21.HM00073	20.21	94.50	252.93	2.46	5.07	0.16	36.00	R5: 0.5/4.5...875µm
05KB21.HM00073	14.05	61.28	223.55	3.42	7.43	0.32	18.00	R5: 0.5/4.5...875µm
05KB21.HM00073	8.97	38.32	172.03	4.26	6.00	0.32	36.00	R5: 0.5/4.5...875µm
05KB21.HM00073	6.21	24.60	136.68	5.30	4.91	0.52	18.00	R5: 0.5/4.5...875µm
05KB21.HM00073	4.42	16.66	64.31	3.59	6.21	1.01	18.00	R5: 0.5/4.5...875µm
05KB21.HM00073	15.63	77.69	220.48	2.64	4.37	0.16	18.00	R5: 0.5/4.5...875µm

Figure 4-24 Pressure and feed rate velocity titration curves of the KB21S (SRM).

By analyzing the pressure and feed rate titration curves shown in Figure 4-24 it is possible to conclude that pressures above 0.5 bar are too high as the PSD of the SRM is shifted to the left, what could indicate that API particles are broken during the analysis at high pressures.

In order to evaluate if the feed rate velocity had a significant influence on the PSD curve, other tests were made using a feed rate of 36 mm/s. In Figure 4-24 can be observed the PSD histograms obtained at different feed rates.

After comparing with the SEM images (bigger particles of 270 µm), the ideal conditions for the analysis for [05KB21S.HM00073] were considered 0.2 bar at 18 mm/s using the R5 lens. The PSD curve under the selected conditions is represented in Figure 4-25.

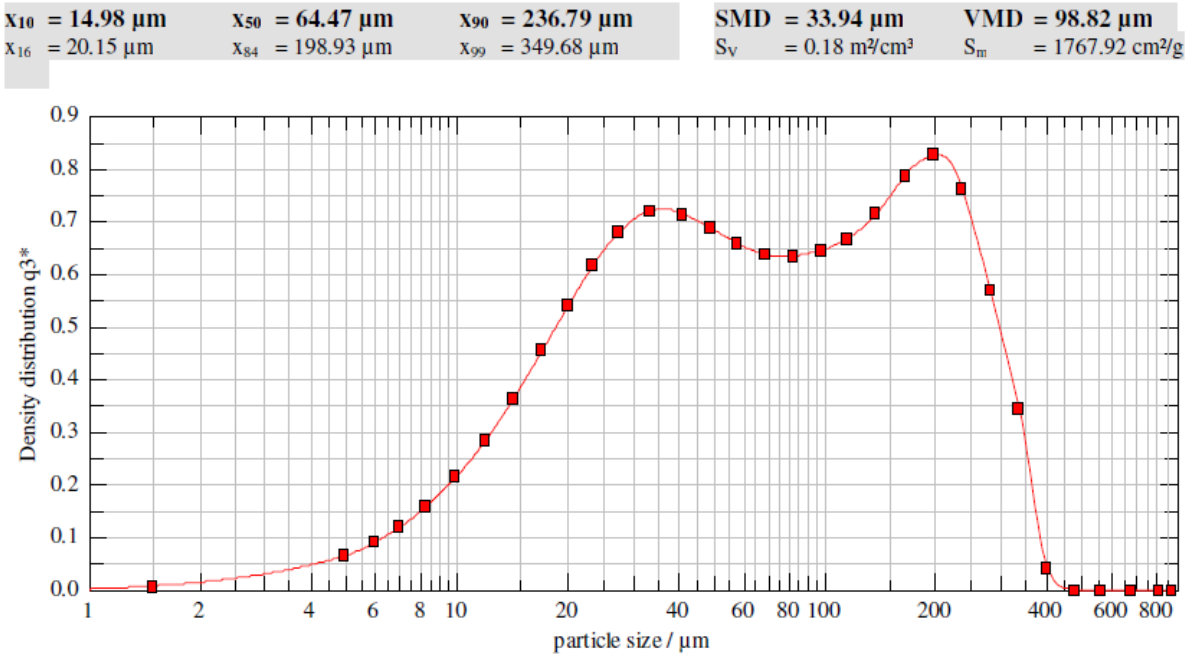


Figure 4-25 PSD results of dry dispersion for the SRM.

A solution of SRM in an appropriate solvent was prepared and processed by spray drying.

The resulting product [06KB21S.SCS059.P1SS] after the mentioned particle engineering process is depicted in Figure 4-26.

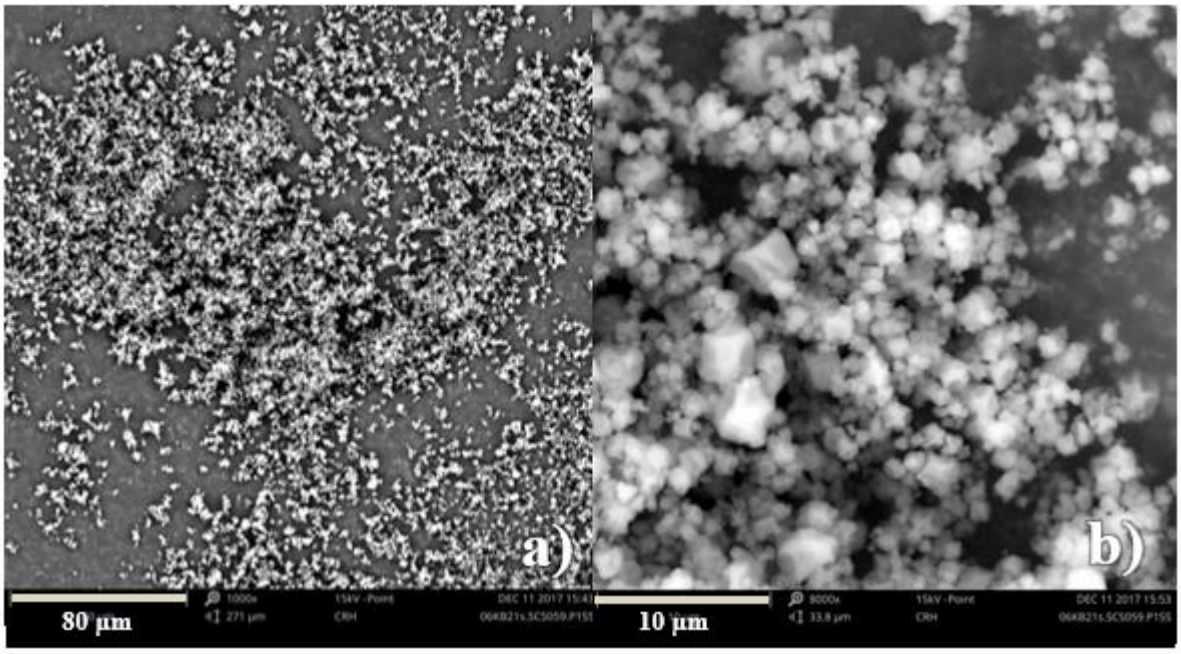
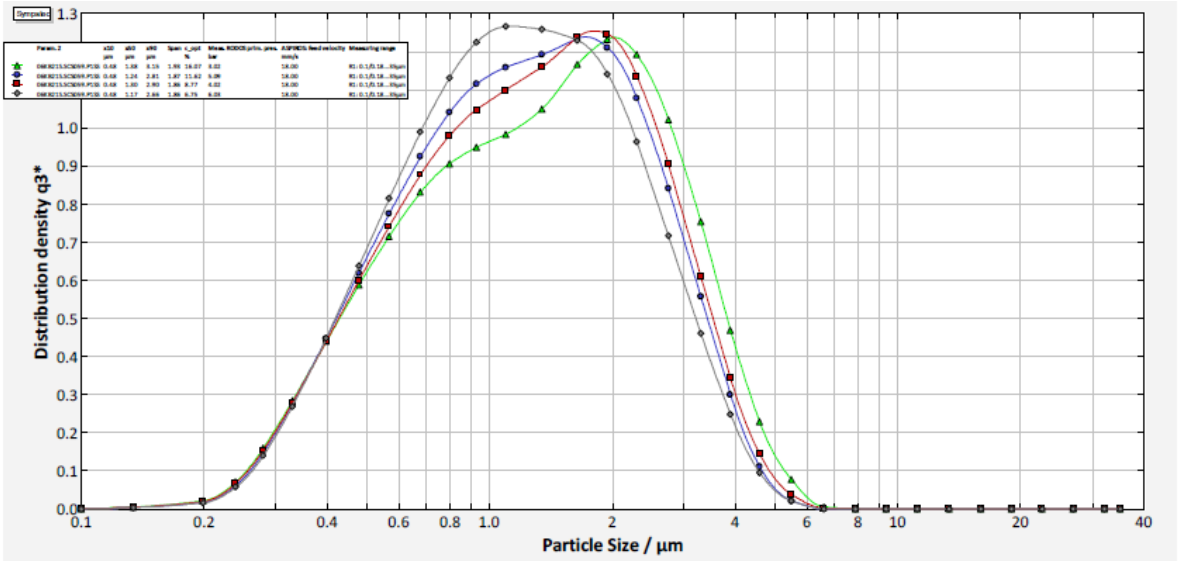


Figure 4-26 a) Product after spray drying at a magnification of 1000x and b) Product after spray drying at a magnification of 8000x.

The particles obtained exhibit round shape and particle size ranging from 0.5-4 µm

The pressure titration curves for the PSD analysis of [06KB21S.SCS059.P1SS] on Sympatec are shown in Figure 4-27.



Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
06KB21S.SCS059.P1SS	0.48	1.38	3.15	1.93	16.07	3.02	18.00	R1:0.1/0.18...35µm
06KB21S.SCS059.P1SS	0.48	1.24	2.81	1.87	11.62	5.09	18.00	R1:0.1/0.18...35µm
06KB21S.SCS059.P1SS	0.48	1.30	2.90	1.86	8.77	4.02	18.00	R1:0.1/0.18...35µm
06KB21S.SCS059.P1SS	0.48	1.17	2.66	1.86	6.75	6.03	18.00	R1:0.1/0.18...35µm

Figure 4-27 Pressure titration curves for the PSD analysis of [06KB21S.SCS059.P1SS]

The ideal conditions for the analysis of [06KB21S.SCS059.P1SS] were established at 5 bar at 18 mm/s using the R1 lens, as the SEM pictures revealed the absence of particles with a size bigger than 5 µm.

Since the API particles after spray drying are quite small (dv90<5µm), the pressures tested were higher than for the SRM. Smaller particles have a higher surface area and therefore tend to form aggregates. For this reason, higher pressures during the analysis are needed to ensure that the particles are measured as individual particles and not as agglomerates, what would lead to an inaccurate PS determination.

The PSD curve at the selected conditions is represented in Figure 4-28.

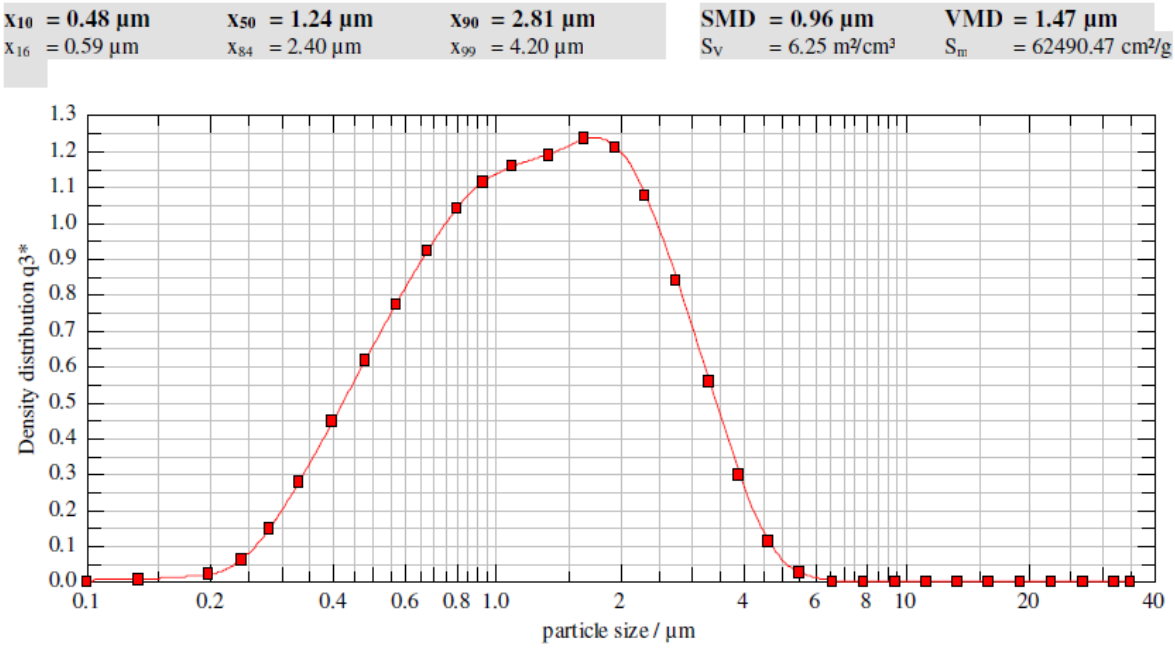


Figure 4-28 Histogram of the dry dispersion of [06KB21S.SCS059.P1SS]

A second fraction was spray dried to assess the impact of the API concentration on the properties of the PS of the API, namely, PS and morphology and residual solvents content.

The starting solution was diluted to half of P1SS in an apporpriate solvent..

The resulting dry fraction [06KB21S.SCS059.P2SS] picture is represented in Figure 4-29.

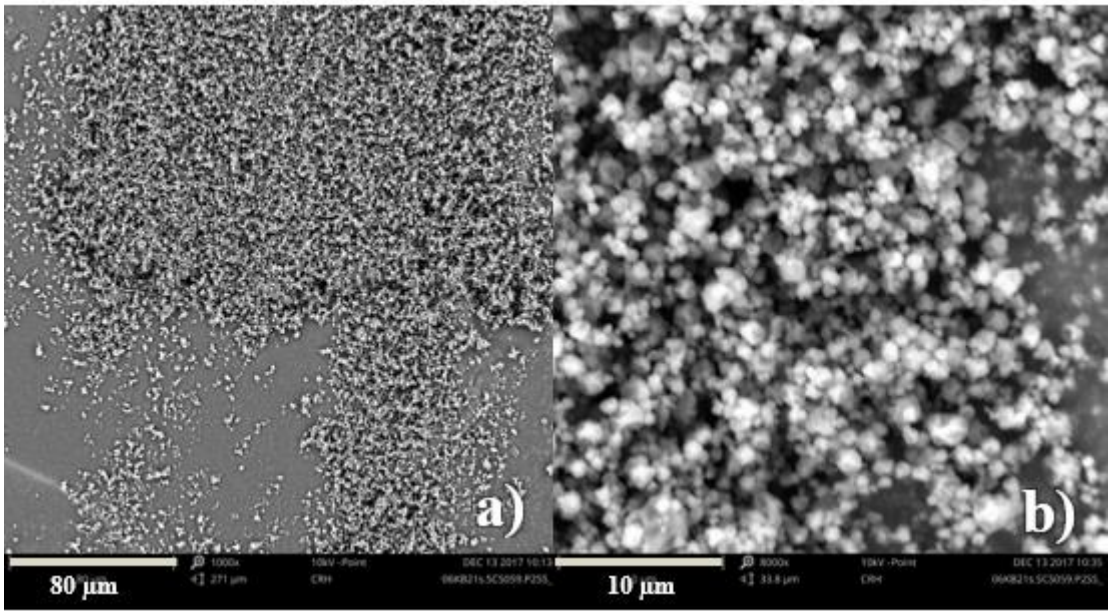
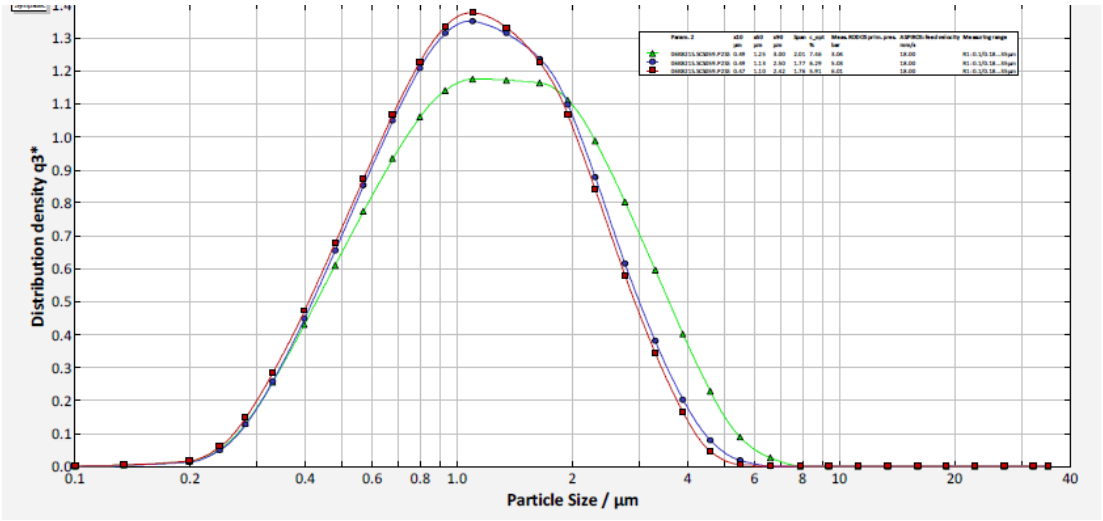


Figure 4-29 a) Product after spray driing at a magnification of 1000x and b) Product after spray drying at aat a magnification of 8000x

As can be seen in Figure 4-29 particles show an overall round shape and a particle size in the range of 0.5 - 4 µm. These results are very similar to what was found in fraction P1SS.

Pressure titration curves were made for the PSD analysis of [06KB21S.SCS059.P2SS] in Sympatec and can be observed in Figure 4-30.



Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
06KB21S.SCS059.P2SS	0.49	1.25	3.00	2.01	7.46	3.04	18.00	R1:0.1/0.18...35µm
06KB21S.SCS059.P2SS	0.49	1.13	2.50	1.77	6.29	5.03	18.00	R1:0.1/0.18...35µm
06KB21S.SCS059.P2SS	0.47	1.10	2.42	1.76	5.91	6.01	18.00	R1:0.1/0.18...35µm

Figure 4-30 Pressure titration curves of [06KB21S.SCS059.P2SS]

The experimental parameters for the analysis of [06KB21S.SCS059.P2SS] were the same as those used to evaluate fraction [06KB21S.SCS059.P1SS]: 5 bar at a feed rate of 18 mm/s using the R1 lens. The PSD curve under the selected conditions is depicted in Figure 4-31.

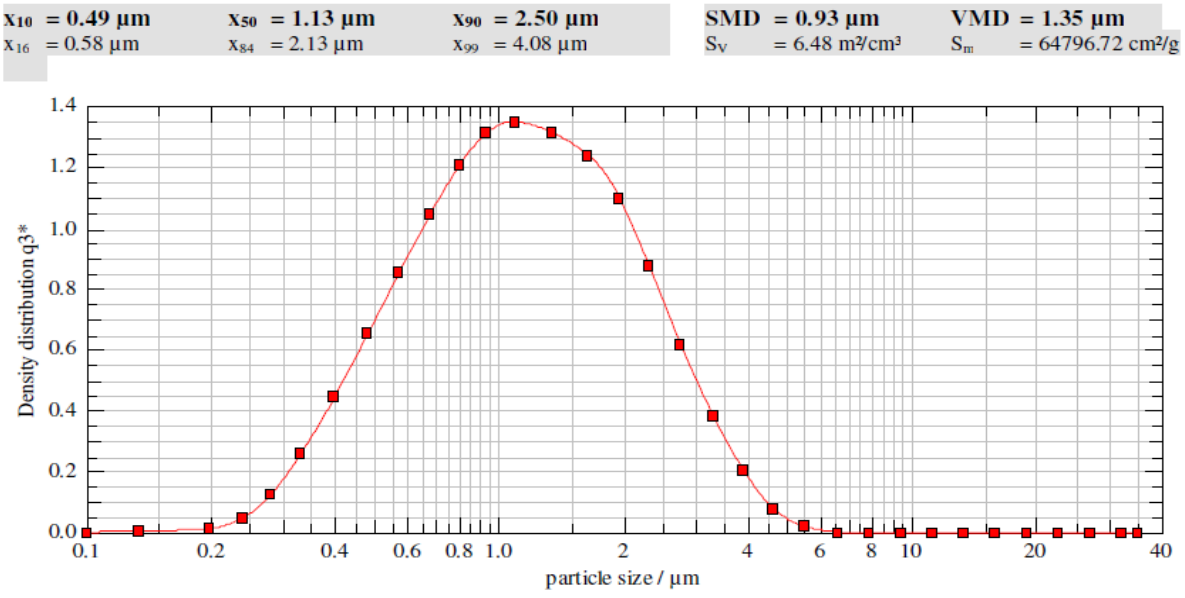
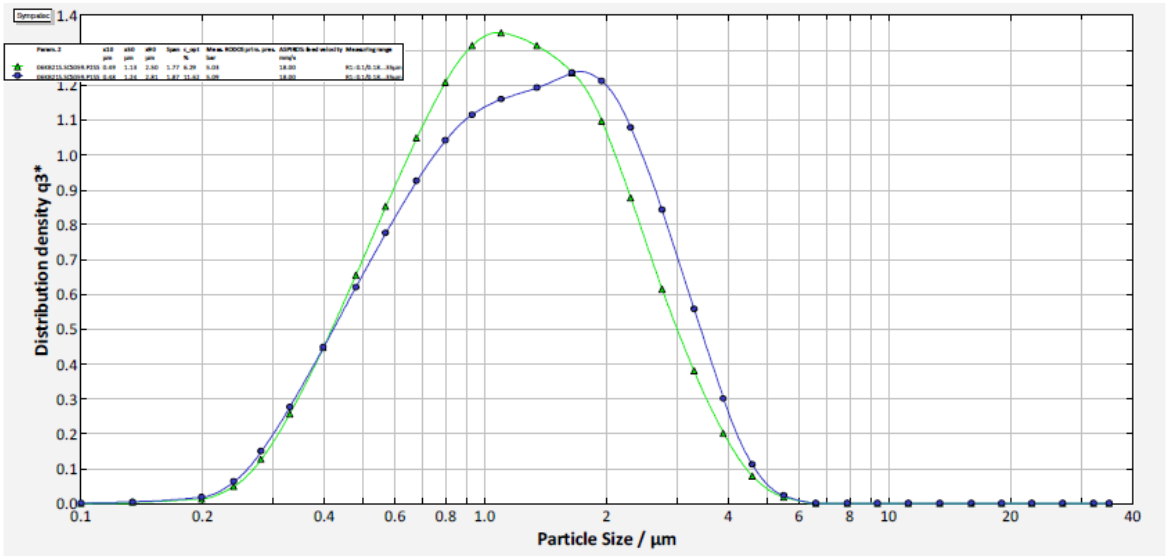


Figure 4-31- Analysis results on dry dispersion for [06KB21S.SCS059.P2SS]

Figure 4-32 show the PSD curves of P1SS and P2SS measured at the same analytical conditions.



Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
06KB21S.SCS059.P2SS	0.49	1.13	2.50	1.77	6.29	5.03	18.00	R1: 0.1/0.18...35µm
06KB21S.SCS059.P1SS	0.48	1.24	2.81	1.87	11.62	5.09	18.00	R1: 0.1/0.18...35µm

Figure 4-32 PSD curves of 06KB21S.SCS059.P1SS and 06KB21S.SCS059.P2SS at the same analytical conditions.

As can be observed PSD results are similar showing that the change in concentration imposed to the API feeding solution did not affect the product PSD.

In Figure 4-33 shown a picture the API before and after the spray drying process.

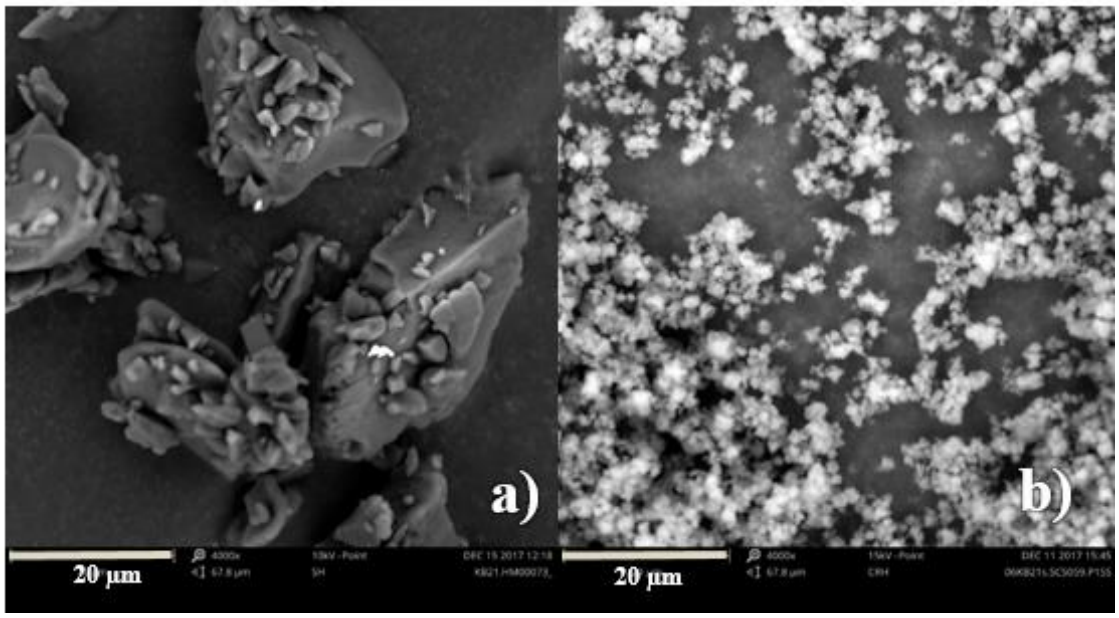
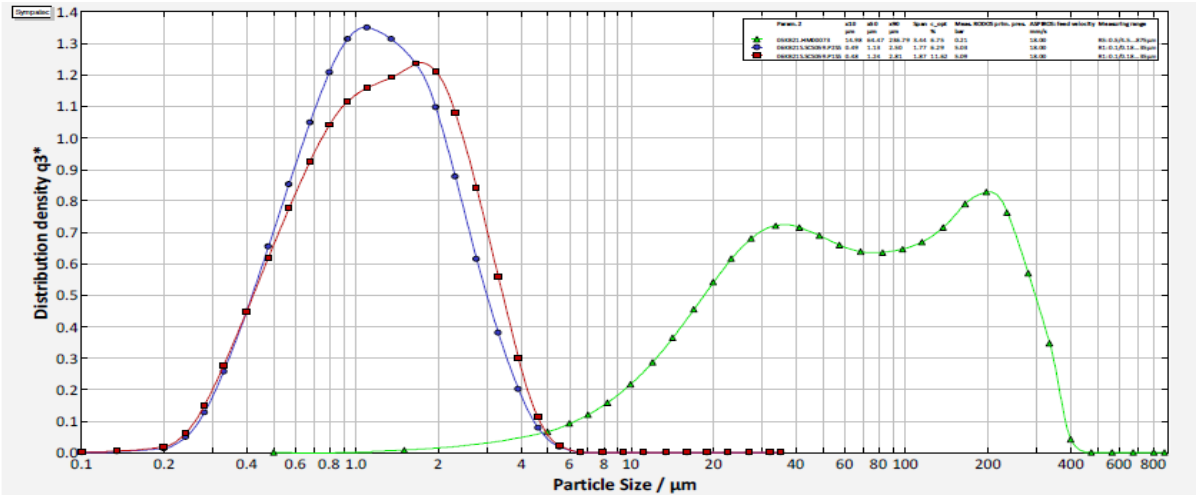


Figure 4-33 a) Product before spray-dried at a magnification of 4000x; b) Product after spray-dried at a magnification of 4000x

As it can be seen, the particle size and morphology of the API suffers a great change after the spray drying process, meeting the PSD requirement established, i.e. a d(0.9) lower than 4 µm.

Figure 4-34 shows the PSD of the SRM and the PSD of the API obtained before and after the spray drying process.



Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
05KB21.HM00073	14.98	64.47	236.79	3.44	6.75	0.21	18.00	R5: 0.5/4.5...875 µm
06KB21S.SCS059.P2SS	0.49	1.13	2.50	1.77	6.29	5.03	18.00	R1: 0.1/0.18...35 µm
06KB21S.SCS059.P1SS	0.48	1.24	2.81	1.87	11.62	5.09	18.00	R1: 0.1/0.18...35 µm

Figure 4-34 Overlay of PSD of SRM and KB21S after being processed by spray drying.

In summary, the process was successful in both fractions, as the PS target was met. SEM analysis performed along the process agreed with particle size assessment carried out in Sympatec, validating the dry-dispersion method.

4.3 Product Characterization

At Hovione, product characterization plays an important role in pharmaceutical development and includes solid-state characterization physical and chemical stability evaluation, assay and related substances determination, particle size and morphology assessment.

In the case of product presented next, the PSD, morphology and dissolution time were evaluated. A comparison of pilot scale (validation) and lab scale batches was carried out to assess the impact of the scale up process.

4.3.1. GD11

GD11 is Hovione's internal code of a pharmaceutical excipient.

The goal of this study was to determine if production (GMP) and laboratory (non-GMP) batches differ regarding particle size and morphology. For this purpose, SEM and particle size analysis were performed. The particle size determination was carried out in Sympatec.

The results of the SEM analysis are illustrated in Figure 4-35.

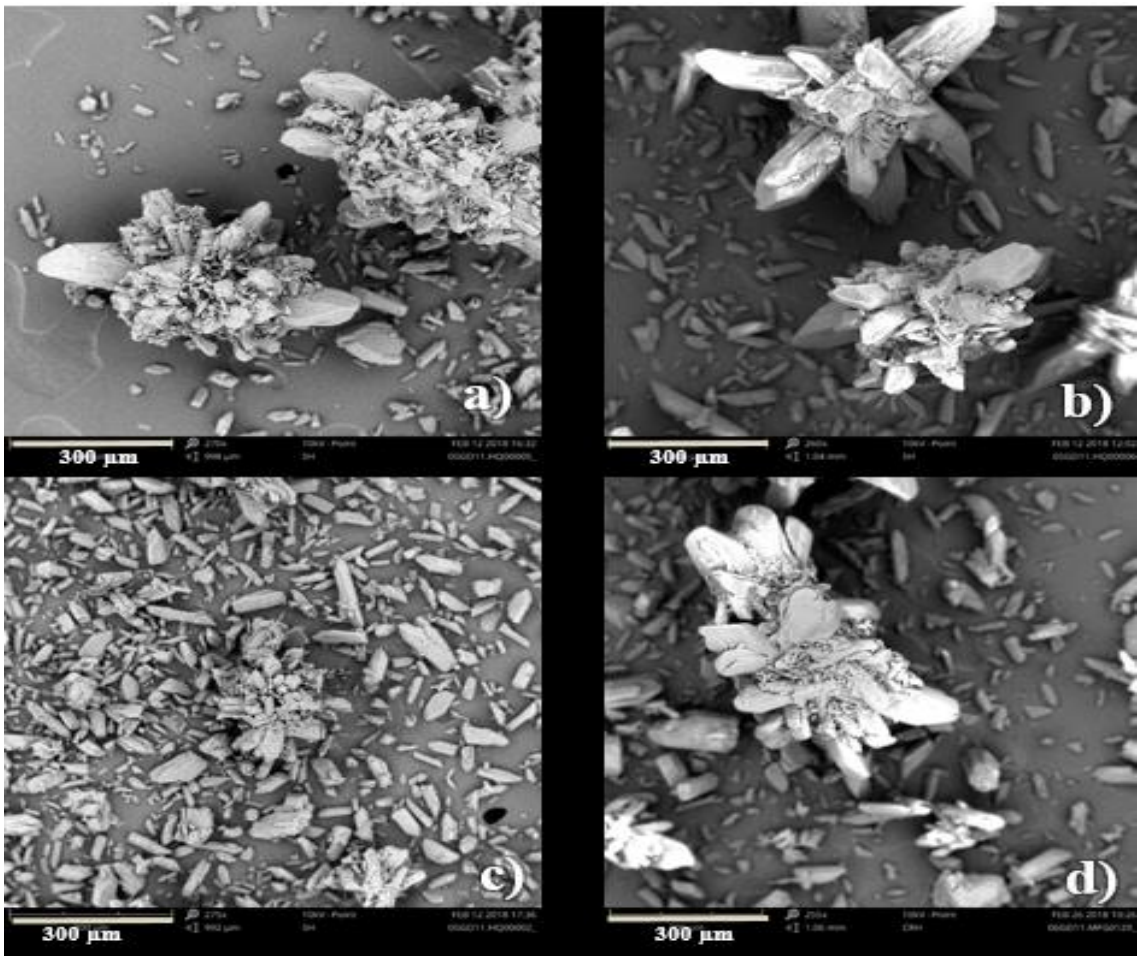
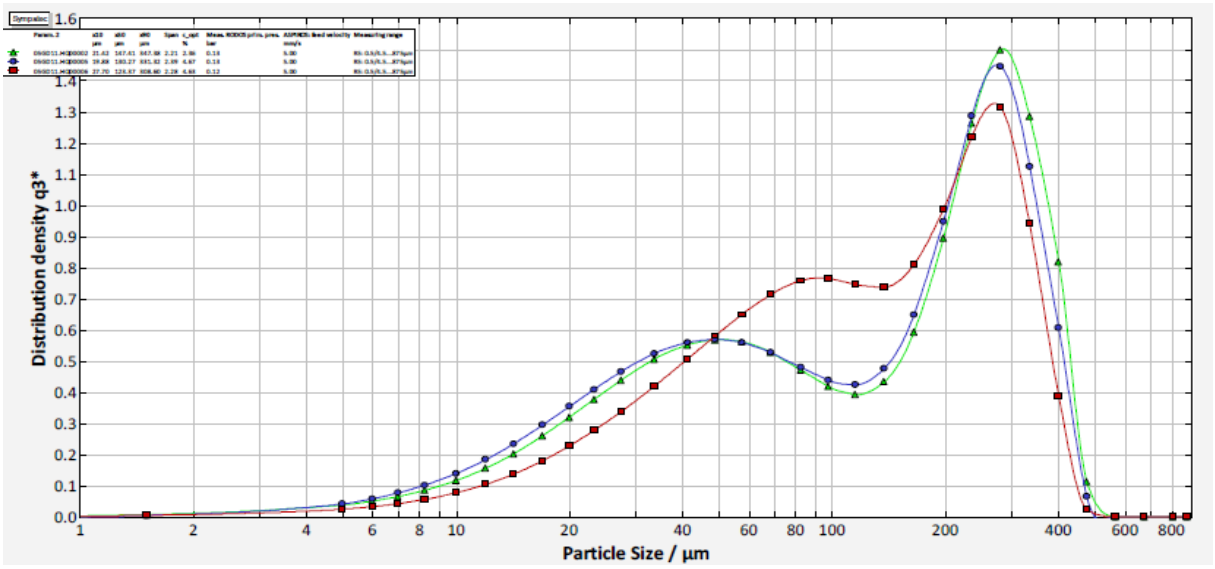


Figure 4-35 SEM images of a) [05GD11.HQ00002]; b) [05GD11.HQ00005]; c) [05GD11.HQ00006] and d) [06GD11.MFG0129].

As can be observed all batches show the presence of particles with similar morphology and particle size. It can be noted that this product exhibits two different populations regarding particle size: coarser particles of size of about 400 µm and a population of smaller particles that agglomerate around the bigger ones. It is interesting to note that this observation is common to both production and laboratory scale batches.

[GD11] PSD determination was carried using a dry dispersion method in Sympatec. The method development is described in detail in chapter 3.

In Figure 4-36 are presented the PSD curves of three production batches studied under the analytical conditions of 0.1 bar, feed rate of 5 mm/s using the R5 lens.

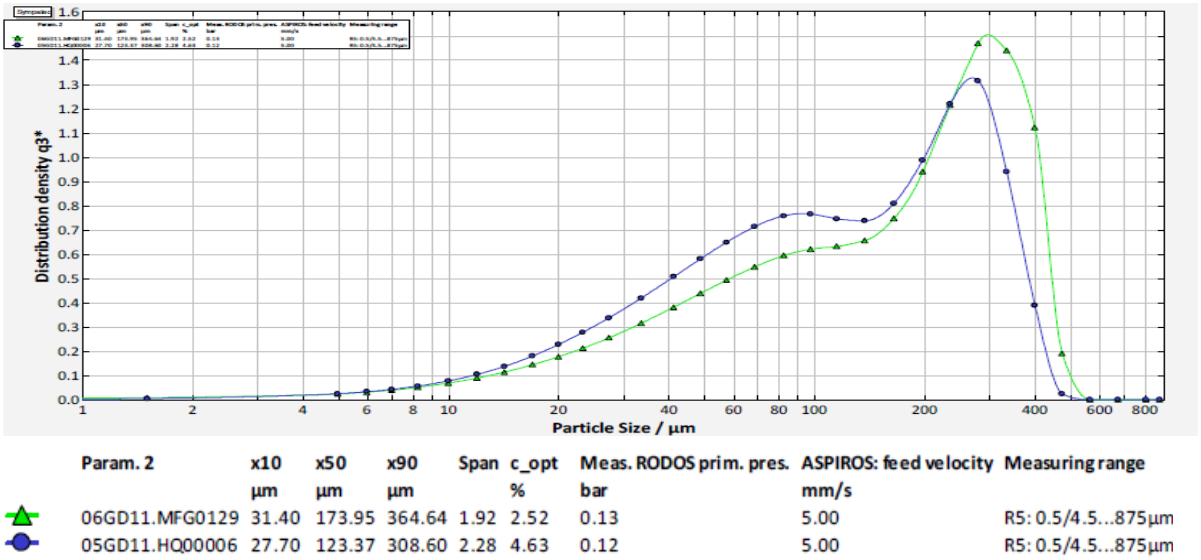


Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
05GD11.HQ00002	21.42	147.41	347.38	2.21	2.36	0.13	5.00	R5: 0.5/4.5...875µm
05GD11.HQ00005	19.88	130.27	331.32	2.39	4.67	0.13	5.00	R5: 0.5/4.5...875µm
05GD11.HQ00006	27.70	123.37	308.60	2.28	4.63	0.12	5.00	R5: 0.5/4.5...875µm

Figure 4-36 PSD histograms of batches [05GD11.HQ00002], [05GD11.HQ00005] and [05GD11.HQ00006]

As can be seen in Figure 4-36 the three batches under evaluation exhibit similar PSD curves. Two different particle populations are clearly identified; coarser particles of 200-400 µm size and smaller particles ranging from 10-100 µm on the left. This PS histogram is coherent with SEM observations (Figure 4-35).

Figure 4-37 shows the PSD curves of one the production batch [05GD11.HQ00006] and the laboratory batch [05GD11.MFG0129]. The analytical conditions were set at 0.1 bar, 5 mm/s feed rate using the R5 lens.



Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
06GD11.MFG0129	31.40	173.95	364.64	1.92	2.52	0.13	5.00	R5: 0.5/4.5...875µm
05GD11.HQ00006	27.70	123.37	308.60	2.28	4.63	0.12	5.00	R5: 0.5/4.5...875µm

Figure 4-37 PSD histograms of batch a production batch [05GD11.HQ00006] and a non-GMP laboratory batch [06GD11.MFG0129]

The results obtained show that PSD of the GMP (production) and non-GMP (laboratory) batches is similar. This fact demonstrate that the scale up process was successful in terms of yielding a product with similar particle size properties and performance.

In Table 4-13 are compiled the PSD results of the four batches evaluated at the same analytical conditions (pressure of 0.1 bar, feed rate of 5 mm/s, R5 lens).

Table 4-13 PS data of production batches [05GD11.HQ00002] [05GD11.HQ00002] [05GD11.HQ00002] and laboratory batch [05GD11.MFG0129]

Batches	**d(0.1) (µm)**	**d(0.5) (µm)**	**d(0.9) (µm)**
[05GD11.HQ00002]	21.4	147.4	347.4
[05GD11.HQ00005]	19.9	130.3	331.3
[05GD11.HQ00006]	27.7	123.4	308.6
[06GD11.MFG0129]	31.4	173.9	364.6
Average	23	133.7	329.1
SD	3.38	10.09	15.92
RSD (%)	15	8	5

In Table 4-13 are showed the average values of d(0.1), d(0.5) and d(0.9) as well as the respective standard deviation. As can be observed there is a good agreement regarding the three particle size parameters (d(0.1), d(0.5) and d(0.9). The RSD is below 10% on d(0.5) and d(0.9) and 15% on d(0.1). The higher value found for the RSD of d(0.1) may be explained by the lower resolution of R5 lens regarding the smaller particle sizes.

In parallel, an additional study was performed to evaluate the dissolution time of the three GMP batches, one non-GMP batch and one micronized batch. The results of this study and process data are described in detail in Appendix 7.VII.

5. Conclusions

Depending on the administration route, API's performance may be highly influenced by the particle size. Yet, the determination of PSD is difficult and complex due to theoretical and technical aspects.

During the course of this thesis, several products were tested for PSD. Particle engineering techniques employed to process the various products (Wet Polishing and Spray Drying) caused changes in their intrinsic physical properties, namely particle size and morphology.

In order to observe and measure the particles, a scanning electron microcopy (SEM) technique was used. Even though this type of equipment does not report the PSD of the sample, it allows a real time observation of the particle morphology and size. In order to minimize errors the sample under analysis should be representative of the bulk and several sections of the stub pin must be thoroughly observed. The SEM analysis was performed at several magnifications and at different locations of the pin sample holder.

The PSD analysis using Laser diffraction comprised wet and dry dispersion techniques, respectively Malvern and Sympatec. In both equipment (Malvern and Sympatec) a good dispersion must be achieved to obtain correct values of the particle size. Bad dispersions may originate incorrect PSD estimation as a result of the formation of the particle aggregates. Besides equipment and dispersion medium, these two methods differ in the algorithm used to generate PSD data. The wet dispersion method uses Mie theory of light scattering that require prior knowledge of the optical properties of the sample and dispersant medium. In opposition, the dry dispersion uses the Fraunhofer approximation and does not require a previous knowledge of the optical parameters.

For [IH11c] the wet dispersion equipment used was Malvern 3000, due to its higher resolution. It was observed that in the Malvern 3000 and Sympatec, the d(0.1) and d(0.5) values are very similar. d(0.9) shows a higher difference between the two methods , however this difference shows a decrease as the product is further micronized. It is also interesting to note that as the PS of the product is reduced, a higher similarity between the results is observed by both techniques. The PSD targets required by the client for [IH11c] were achieved and the data obtained by SEM, Malvern 3000 and Sympatec showed a good agreement.

For products [ST71c] and [ST52c] it was observed that the Malvern 2000 and Sympatec were in good agreement. However, for [06ST71c.SCS061.P1SS] and [06ST71c.SCS061.P3SS] fractions, the Sympatec reported values were slightly higher than those obtained uisng Malvern 2000.

For [KB21S] the PS analysis was carried out using Sympatec. PS data obtained from Sympatec was confirmed with SEM images performed along the process. The combination of these two techniques, allows the observation and comparison of the particle size and morphology changes of the product before and after the spray drying process.

Another product analyzed in this thesis was the pharmaceutical excipient [GD11]. The particle size of four batches of this excipient was assessed using SEM and Sympatec. Additionally, solubility studies at different temperatures and concentrations were carried out in Crystalline equipment.

It was concluded that there was good agreement among the batches analyzed regarding the three particle size parameters (d(0.1), d(0.5) and d(0.9). The RSD was always lower than 10% on d(0.5) and d(0.9) and 15% on d(0.1). The higher value found for (0.9) may be due by the lower resolution of R5 lens for particle sizes below 4.5 µm.

The differences found in the PSD parameters of the several products analyzed by Malvern and Sympatec may be justified by several facts. Sympatec use the Fraunhofer model that it usually applied to opaque spherical particles whose particle sizes is higher than 50 µm. However, the variety of specific lens set reduces this restriction. The Mie theory used by Malvern equipment, it is not appropriate for fine particulates below 0.1 µm. This is possibly due to the increased sensitivity to changes in the refractive index that occur for particles in this size range. For particles larger than 50 µm, usually the Fraunhofer approximation and Mie theory provide very similar results. For particles in the size range of 2 to 50 µm, the data reported depend on the values of the refractive index of sample and medium.

The SEM analysis provides valuable information as it provides an approximate estimation of the sample particle size, especially regarding the d(0.9) parameter.

The potential breakage of particles during analysis is may be a limitation during PSD determination if an excess of ultrasounds (for wet dispersion techniques) or an excess of pressure (for dry dispersion) is employed. This phenomenon was found to be particular relevant for needle-shaped crystals with particle size above 100 µm.

Since these two laser diffraction techniques use different mathematical models for generating PSD data it is expected that the PSD values reported are not exactly coincident. This means that every PS request should be always reported to a validated method. Generally, that is what happens at Hovione. The clients request product with a specific PSD reporting to a validated method.

6. References

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7. Appendixes

I. Equipment's specifications

a) Scanning Electron Microscope (Phenom ProX)

Table 7-1 Phenom ProX equipment specifications

Equipment specifications	
Light optical magnification range	20 – 135 x
Electron optical magnification range	80 – 130000 x
Resolution	≤ 10 nm
Digital zoom	Max 12 x
Light optical navigation camera	Color
Acceleration voltages	Default: 5 kV, 10 kV and 15kV ; Advanced: adjustable range between 4.8kV and 15 kV imaging and analysis mode
Vacuum modes	Standard mode and charge reduction mode
Detector	BSD and EDS
Sample size	Up to 32 mm
Sample height	Up to 100 mm

b) Malvern Hydro 2000s

Table 7-2 Malvern Hydro 2000s equipment specifications

Equipment specifications	
Dispersion type	Wet
Capacity	50 – 150 ml
Typical applications	Solvent-based suspensions, Pharmaceuticals
Dispersion mechanisms	Continuously variable combined pump/stirrer ; Continuously variable ultrasonics
Modes of operation	Automatic via SOPs ; Manual via computer operating dialogs
Weight	16.8 kg
Dimensions	Width: 348 mm ; Height: 333 mm ; Depth: 365 mm
Power	100 – 120 V / 200/240 V AC, 50/60 Hz, 240 VA
Maximum size of particles	Up to 600 microns, depending on particle shape and density

c) Malvern Mastersizer 3000 Hydro MV

Table 7-3 Malvern Mastersizer 3000 Hydro MVequipment specifications

Equipment specifications	
Pump speed range:	0-3500 rpm
Pump speed accuracy:	+/- 50 rpm
Pump speed resolution:	+/- 10 rpm
Maximum flow rate:	2.0 l/min.
Sonication power & frequency:	40W max, 40kHz (nominal)
Maximum volume:	120ml
Materials in contact with the dispersant, additives and sample:	316 stainless Borosilicate Glass Tygon® R-3603. Viton (cell seal only - perfluoroelastomer upgrade available) PTFE PEEK FEP Titanium Nitride Aluminum (tubing connectors only) Acrylic (splash guard only)
Maximum particle size:	1500 µm
Minimum time between measurements:	Less than 60 seconds
Dimensions (W, D, H):	180mm x 280mm x 300mm
Weight:	5kg

d) Sympatec (dispersion unit RODOS/M)

Table 7-4 RODOS/M unit equipment specifications

Equipment specifications		
Principle	Aerosol jet	Particle – Particle collisions ; Particle – Wall collisions ; Velocity gradients
Dispersion	Pressure	0.1 – 6 bar
	Injector airflow	300 l/min
	Extraction	Streamlined
Feeding	VIBRI funnel dosing	Vibratory feeder: mg – kg
	ASPIROS tube feed	Micro feeder: µg – mg
QA-system	Guarantee	50000 shots: 4 g cement PZ35
Operation	Software-controlled	Standard operating procedures (SOPs)
Application	HELOS laser diffraction measuring ranges	0.1 µm – 3500 µm R1 – R7

e) Sympatec (laser diffraction sensor HELOS)

Table 7-5 HELOS unit equipment specifications

Equipment specifications		
Sensor:	HELOS	BR: 0.1 µm - 875 µm KR: 0.1 µm - 8750 µm VARIO: 0.1 µm - 8750 µm
Principle:	Laser diffraction	l = 632,8 nm
Dispersion:	Adaptable modules	Aerodispersion, sprays, suspensions, emulsions
Measurement:	Multi-element detector frequency	31 semi-circular elements 2000/s, permanent autofocus
Evaluation:	Fraunhofer Enhanced Evaluation (FREE)	Mie Extended Evaluation (MIEE) as an option
Ranges:	Optical modules	R1 to R8
Performance:	Accuracy, repeatability, comparability, x_{10}, x_{50}, x_{90}	s < 2% mean rel. SD to absolute value s < 0.04% typical (repeated sample) s < 0.3% typical (riffled sample) s < 1% mean rel. SD /Dx/ < 2.5% rel max. deviation < 5% rel. deviation in the submicron range
Operation:	WINDOX v5.9.1.2 software	WINDOWS 7 / Vista Prof. / XP Prof.
Applications:	laser power protection class/type beam diameter ($1/e^2$)	5 mW 3R/IP40 R1 & R2 : 2.2 mm R3-R5: 13 mm R6 & R7: 26 mm R8: 35mm
QA-system	Certification reference material validation	Standard test procedure SiC - F1200 (x_{50} = 4.5 µm) SiC - P600 (x_{50} = 27µm) SiC - P80 (x_{50} = 260 µm) SiC - P50 (x_{50} =430 µm) FDA approved

f) Sympatec (Accessory ASPIROS)

Table 7-6 ASPIROS unit equipment specifications

Equipment specifications	
Applications	Controlled dosing of dry powders as accessory to RODOS, RODOS/m and OASIS
Sample quantity	< 1 mg to about 1g
Measuring range	0.1 µm to 875 µm
Dosing	Speed controlled
Sample preparation	Glass tubes
Capped tube	For safe handling and processing
Bar-code	Adhesive bar-code label for identification and parameter set-up prior to measurement supports all standard codes, i.e. CODE 39, Interleaved 2 of 5, CODABAR, Code 128
Operation	Manual feeding of the sample glass tube containing the sample into the transport sled, operation starts only if cover is closed and locked; Sample glass detection; Automatic un-capping
Simplified cleaning	All parts in contact with the sample are easily removable
Control	By software via RS485 Interface or manually via panel with alphanumeric display; Supported by WINDOX 5 software

II. Malvern PSD analysis results

a) Malvern 2000 PSD curve for [06IH11c.HQ00010.P6SS]

Particle Name: (1.52, 0.01)	**Accessory Name:** Hydro 2000S (A)	**Delay between measurments:** 0 s	**Analysis model:** General purpose	**Sensitivity:** Normal
Particle RI: 1.520	**Absorption:** 0.01		**Size range:** 0.020 to 2000.000 um	**Obscuration:** 5.85 %
Dispersant Name: Acetone	**Dispersant RI:** 1.360	**Stirrer Speed:** 2100 R...	**Weighted Residual:** 0.782 %	**Residual:** 0.254 %

Concentration: 0.0018 %Vol	**Span :** 1.699	**Mode:** 2.954	**Uniformity:** 0.52	**Result units:** Volume
Specific Surface Area: 2.83 m²/g	**Surface Weighted Mean D[3,2]:** 2.120 um	**Vol. Weighted Mean D[4,3]:** 3.130 um	**Measurement integration time:** 10000 mS	

d(0.1): 1.069 um **d(0.5):** 2.736 um **d(0.9):** 5.718 um

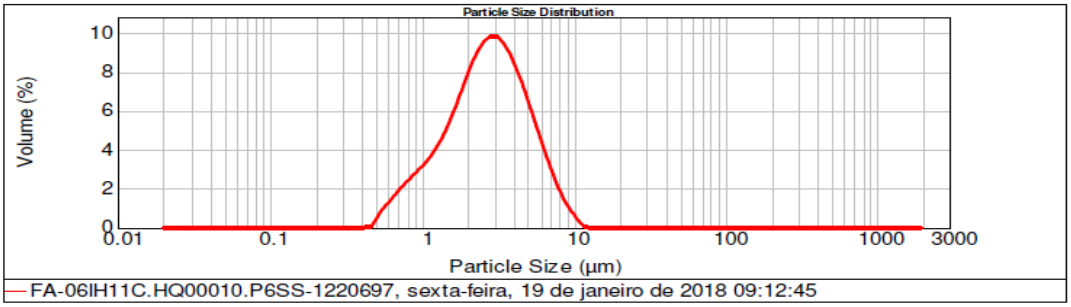


Figure 7-1 PSD result of the average value of the wet dispersion for [06IH11c.HQ00010.P6SS].

b) Malvern 2000 PSD curve for [06IH11c.HQ00010.P8SS]

Particle Name: (1.52, 0.01)	**Accessory Name:** Hydro 2000S (A)	**Delay between measurments:** 0 s	**Analysis model:** General purpose	**Sensitivity:** Normal
Particle RI: 1.520	**Absorption:** 0.01		**Size range:** 0.020 to 2000.000 um	**Obscuration:** 5.14 %
Dispersant Name: Acetone	**Dispersant RI:** 1.360	**Stirrer Speed:** 2100 R...	**Weighted Residual:** 0.685 %	**Residual:** 0.554 %

Concentration: 0.0014 %Vol	**Span :** 1.883	**Mode:** 2.418	**Uniformity:** 0.587	**Result units:** Volume
Specific Surface Area: 3.82 m²/g	**Surface Weighted Mean D[3,2]:** 1.572 um	**Vol. Weighted Mean D[4,3]:** 2.437 um	**Measurement integration time:** 10000 mS	

d(0.1): 0.772 um **d(0.5):** 2.054 um **d(0.9):** 4.639 um

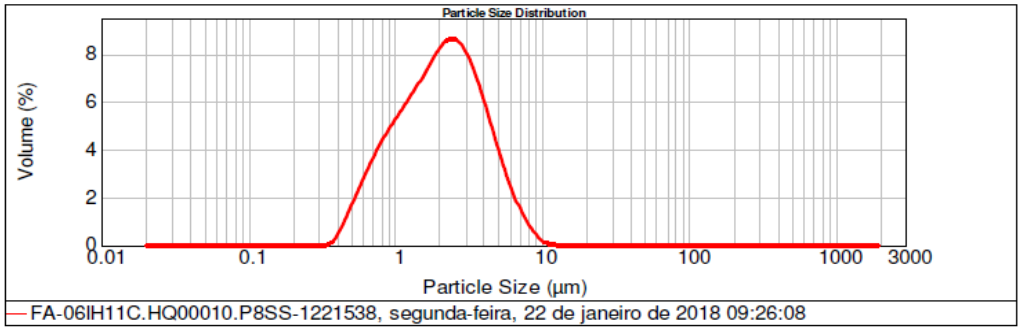


Figure 7-2 PSD result of the average value of the wet dispersion for [06IH11c.HQ00010.P6SS].

c) Malvern 3000 PSD curves for [06IH11c.HQ00010.P6SS]

Analysis		Result	
Particle Name	IH11	**Concentration**	0.0010 %
Particle Refractive Index	1.650	**Span**	1.998
Particle Absorption Index	1.000	**Uniformity**	0.616
Particle Refractive Index Blue	1.650	**Specific Surface Area**	3613 m²/kg
Particle Absorption Index Blue	1.000	**D [3;2]**	1.58 µm
Dispersant Name	Acetone	**D [4;3]**	2.60 µm
Dispersant Refractive Index	1.360	**Dv (10)**	0.738 µm
Analysis Model	General Purpose	**Dv (50)**	2.17 µm
Single Result Mode?	No	**Dv (90)**	5.07 µm
Weighted Residual	0.75 %	**Mode**	2.50 µm
Laser Obscuration	5.31 %	**Result Units**	Volume
Residual	0.71 %	**Sample Measurement Time**	10.00 s
Stirrer Speed Achieved	3500 rpm	**Laser Power**	80.22 %
Accessory Name	Hydro MV		
Analysis Sensitivity	Normal		
Particle Density	1.05 g/cm³		

Frequency (compatible)

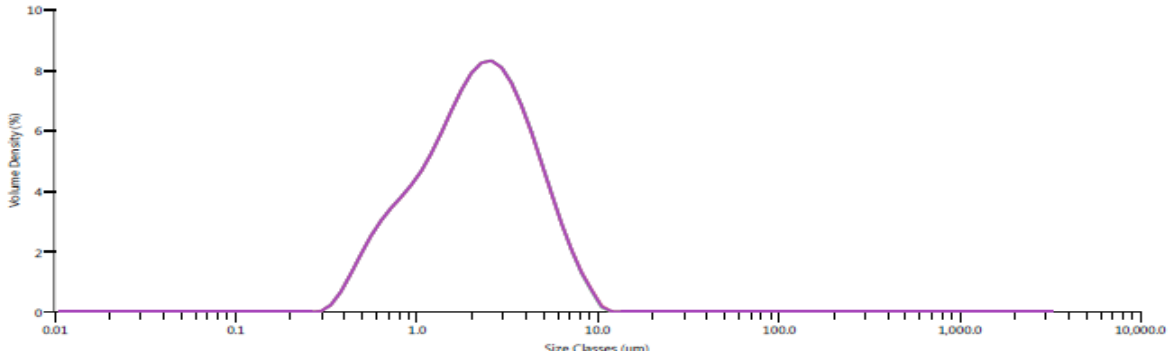


Figure 7-3 PSD result of the average value of the wet dispersion for [06IH11c.HQ00010.P6SS].

d) Malvern 3000 PSD curves for [06IH11c.HQ00010.P8SS]

Analysis		Result	
Particle Name	IH11	**Concentration**	0.0008 %
Particle Refractive Index	1.650	**Span**	1.867
Particle Absorption Index	1.000	**Uniformity**	0.580
Particle Refractive Index Blue	1.650	**Specific Surface Area**	4646 m²/kg
Particle Absorption Index Blue	1.000	**D [3;2]**	1.23 µm
Dispersant Name	Acetone	**D [4;3]**	1.90 µm
Dispersant Refractive Index	1.360	**Dv (10)**	0.599 µm
Analysis Model	General Purpose	**Dv (50)**	1.62 µm
Single Result Mode?	No	**Dv (90)**	3.63 µm
Weighted Residual	1.18 %	**Mode**	2.04 µm
Laser Obscuration	5.30 %	**Result Units**	Volume
Residual	0.72 %	**Sample Measurement Time**	10.00 s
Stirrer Speed Achieved	3500 rpm	**Laser Power**	79.75 %
Accessory Name	Hydro MV		
Analysis Sensitivity	Normal		
Particle Density	1.05 g/cm³		

Frequency (compatible)

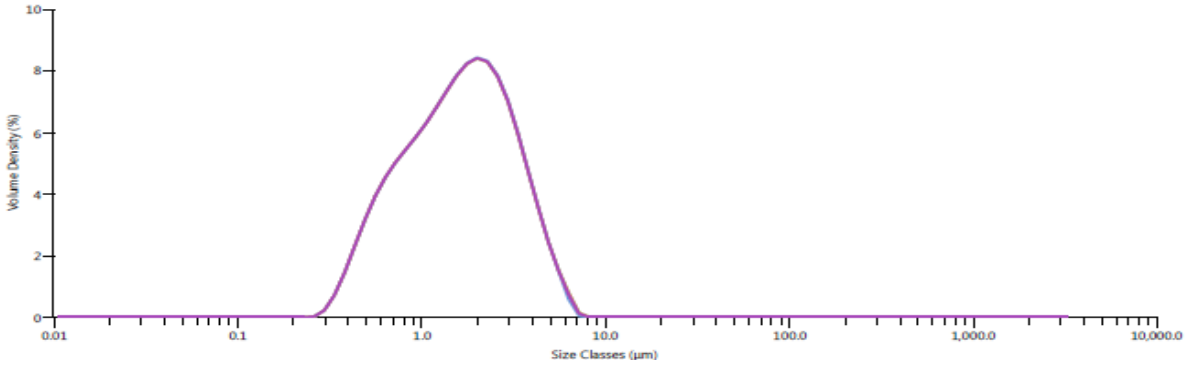


Figure 7-4 PSD result of the average value of the wet dispersion for [06IH11c.HQ00010.P8SS].

e) Malvern 3000 PSD curves for [06IH11c.HQ00011.P3SS]

Analysis		Result	
Particle Name	IH11	**Concentration**	0.0017 %
Particle Refractive Index	1.650	**Span**	2.779
Particle Absorption Index	1.000	**Uniformity**	0.880
Particle Refractive Index Blue	1.650	**Specific Surface Area**	2575 m²/kg
Particle Absorption Index Blue	1.000	**D [3;2]**	2.22 µm
Dispersant Name	Acetone	**D [4;3]**	4.84 µm
Dispersant Refractive Index	1.360	**Dv (10)**	0.952 µm
Analysis Model	General Purpose	**Dv (50)**	3.43 µm
Single Result Mode?	No	**Dv (90)**	10.5 µm
Weighted Residual	0.47 %	**Mode**	3.85 µm
Laser Obscuration	6.35 %	**Result Units**	Volume
Residual	0.60 %	**Sample Measurement Time**	10.00 s
Stirrer Speed Achieved	3500 rpm	**Laser Power**	79.83 %
Accessory Name	Hydro MV		
Analysis Sensitivity	Normal		
Particle Density	1.05 g/cm³		

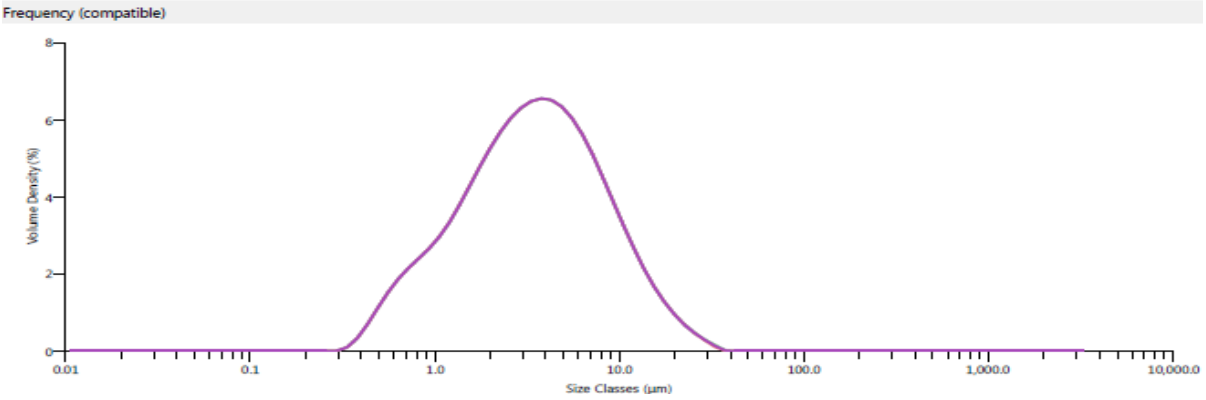


Figure 7-5 PSD result of the average value of the wet dispersion for [06IH11c.HQ00011.P3SS].

f) Malvern 3000 PSD curves for [06IH11c.HQ00011.P5SS]

Analysis		Result	
Particle Name	IH11	**Concentration**	0.0015 %
Particle Refractive Index	1.650	**Span**	2.045
Particle Absorption Index	1.000	**Uniformity**	0.642
Particle Refractive Index Blue	1.650	**Specific Surface Area**	4785 m²/kg
Particle Absorption Index Blue	1.000	**D [3;2]**	1.19 µm
Dispersant Name	Acetone	**D [4;3]**	1.91 µm
Dispersant Refractive Index	1.360	**Dv (10)**	0.581 µm
Analysis Model	General Purpose	**Dv (50)**	1.56 µm
Single Result Mode?	No	**Dv (90)**	3.76 µm
Weighted Residual	1.11 %	**Mode**	1.96 µm
Laser Obscuration	10.70 %	**Result Units**	Volume
Residual	0.63 %	**Sample Measurement Time**	10.00 s
Stirrer Speed Achieved	3500 rpm	**Laser Power**	79.83 %
Accessory Name	Hydro MV		
Analysis Sensitivity	Normal		
Particle Density	1.05 g/cm³		

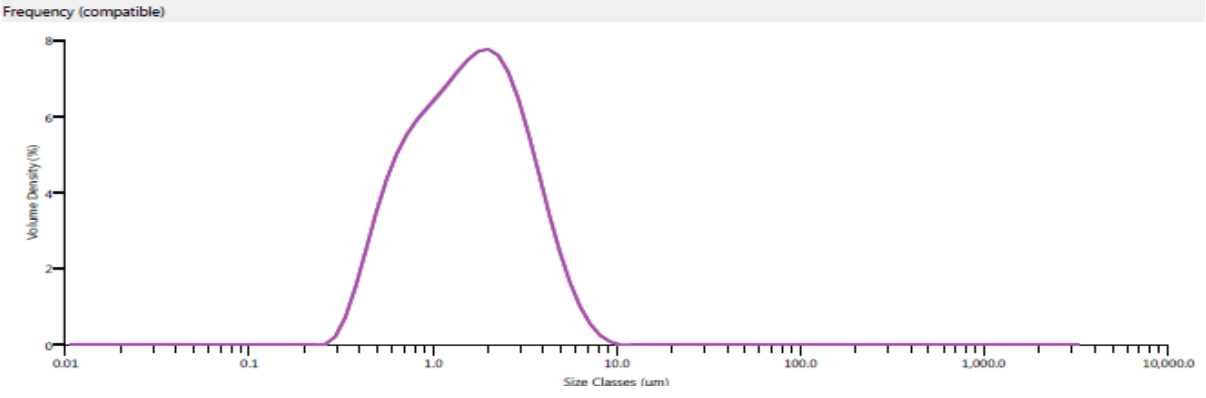
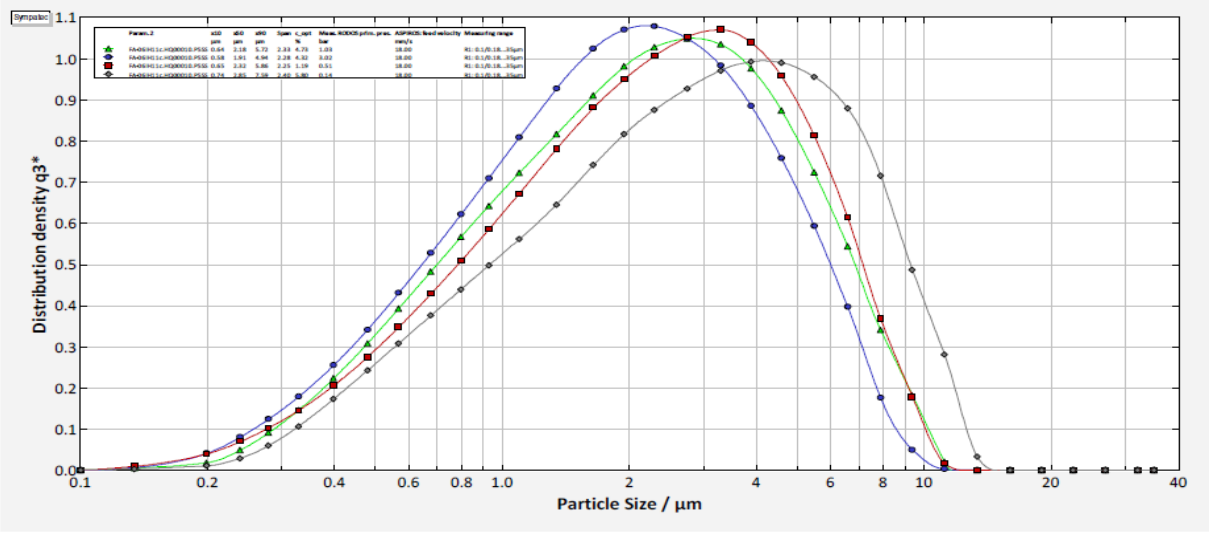


Figure 7-6 PSD result of the average value of the wet dispersion for [06IH11c.HQ00011.P5SS].

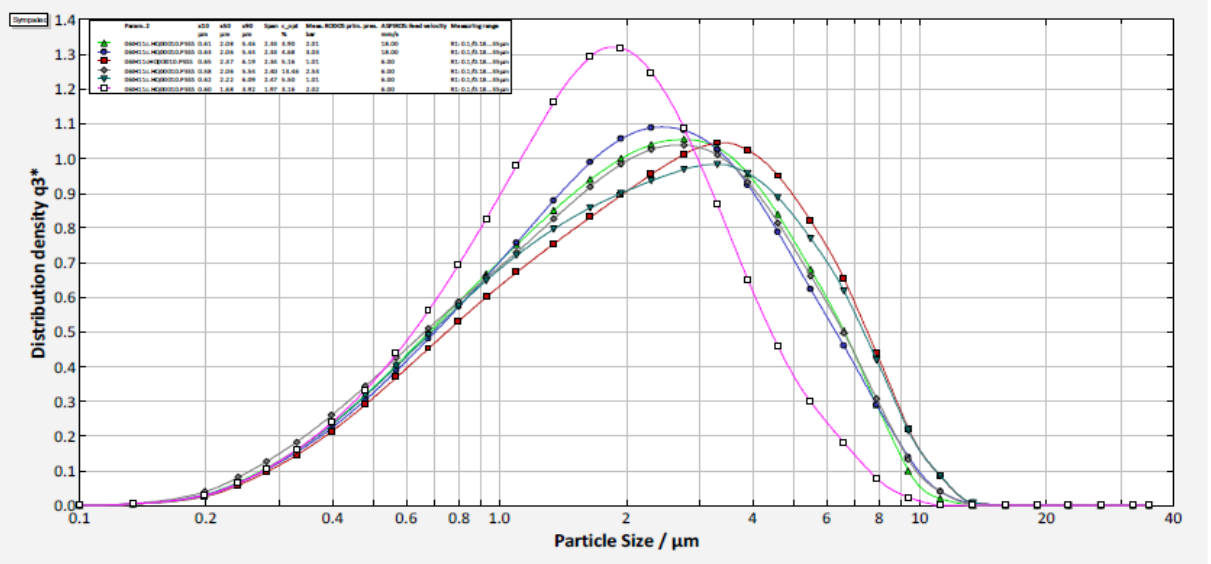
III. [IH11c] titration curves: Pressure and Feed rate velocity

a) [IH11c] titration curves for [06IH11c.HQ00010.P5SS]



Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
FA-06IH11c.HQ00010.P5SS	0.64	2.18	5.72	2.33	4.73	1.03	18.00	R1: 0.1/0.18...35µm
FA-06IH11c.HQ00010.P5SS	0.58	1.91	4.94	2.28	4.32	3.02	18.00	R1: 0.1/0.18...35µm
FA-06IH11c.HQ00010.P5SS	0.65	2.32	5.86	2.25	1.19	0.51	18.00	R1: 0.1/0.18...35µm
FA-06IH11c.HQ00010.P5SS	0.74	2.85	7.59	2.40	5.80	0.14	18.00	R1: 0.1/0.18...35µm

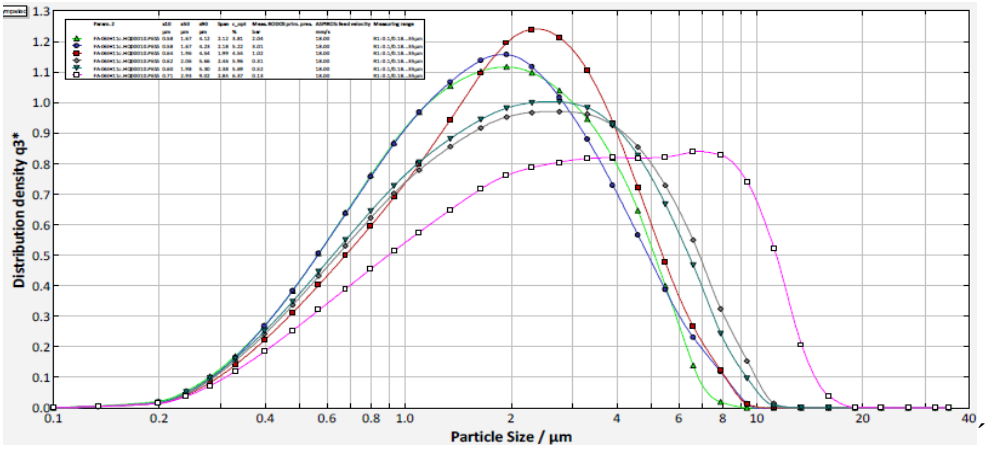
Figure 7-7 Pressure titration curves of [06IH11c.HQ00010.P5SS]



Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
06IH11c.HQ00010.P5SS	0.61	2.08	5.46	2.33	3.90	2.01	18.00	R1: 0.1/0.18...35µm
06IH11c.HQ00010.P5SS	0.63	2.06	5.44	2.33	4.68	3.03	18.00	R1: 0.1/0.18...35µm
06IH11cHQ00010.P5SS	0.65	2.37	6.19	2.34	5.16	1.01	6.00	R1: 0.1/0.18...35µm
06IH11c.HQ00010.P5SS	0.58	2.06	5.54	2.40	13.46	2.53	6.00	R1: 0.1/0.18...35µm
06IH11c.HQ00010.P5SS	0.62	2.22	6.09	2.47	5.50	1.01	6.00	R1: 0.1/0.18...35µm
06IH11c.HQ00010.P5SS	0.60	1.68	3.92	1.97	3.16	2.02	6.00	R1: 0.1/0.18...35µm

Figure 7-8 Feed rate velocity titration curves of [06IH11c.HQ00010.P5SS]

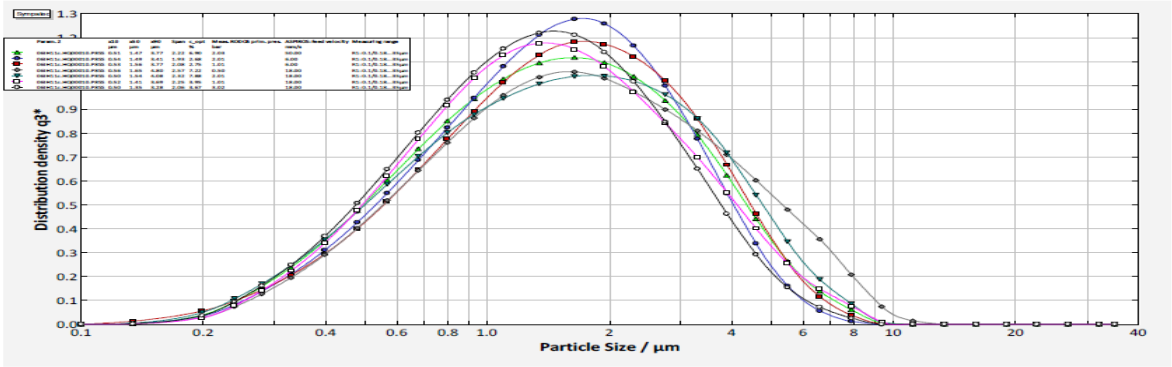
b) [IH11c] titration curves for [06IH11c.HQ00010.P6SS]



Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
FA-06IH11c.HQ00010.P6SS	0.58	1.67	4.12	2.12	3.81	2.04	18.00	R1: 0.1/0.18...35µm
FA-06IH11c.HQ00010.P6SS	0.58	1.67	4.23	2.18	5.22	3.01	18.00	R1: 0.1/0.18...35µm
FA-06IH11c.HQ00010.P6SS	0.64	1.96	4.54	1.99	4.54	1.02	18.00	R1: 0.1/0.18...35µm
FA-06IH11c.HQ00010.P6SS	0.62	2.06	5.66	2.46	5.96	0.31	18.00	R1: 0.1/0.18...35µm
FA-06IH11c.HQ00010.P6SS	0.60	1.98	5.30	2.38	5.69	0.52	18.00	R1: 0.1/0.18...35µm
FA-06IH11c.HQ00010.P6SS	0.71	2.93	9.02	2.84	6.37	0.13	18.00	R1: 0.1/0.18...35µm

Figure 7-9 Pressure and Feed rate velocity titration curves of [06IH11c.HQ00010.P6SS]

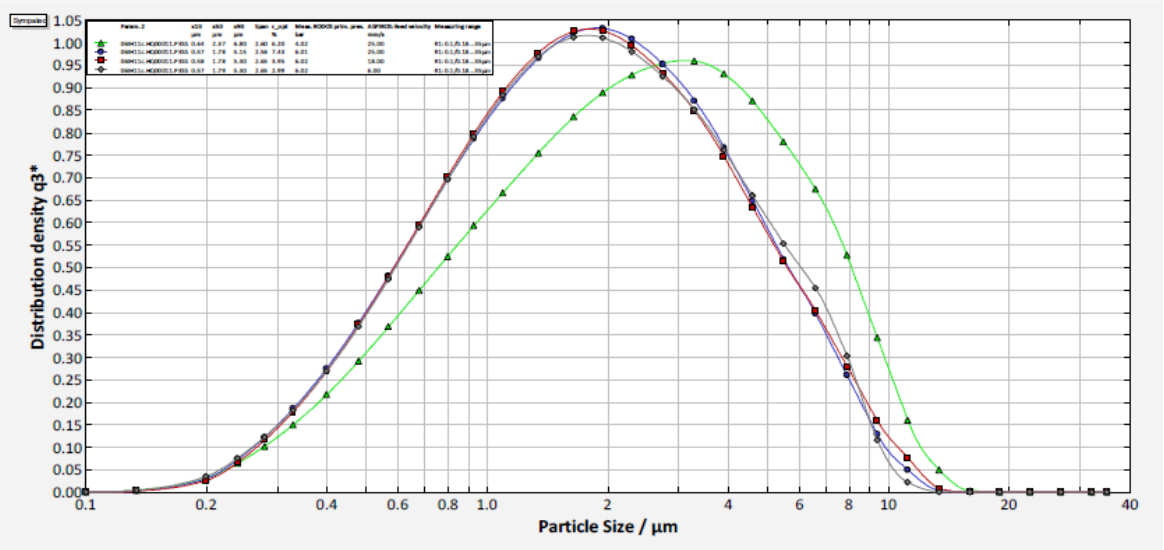
c) [IH11c] titration curves for [06IH11c.HQ00010.P8SS]



Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
06IH11c.HQ00010.P8SS	0.51	1.47	3.77	2.22	6.90	2.03	50.00	R1: 0.1/0.18...35µm
06IH11c.HQ00010.P8SS	0.54	1.49	3.41	1.93	2.68	2.01	6.00	R1: 0.1/0.18...35µm
06IH11c.HQ00010.P8SS	0.53	1.56	3.77	2.08	2.75	1.01	6.00	R1: 0.1/0.18...35µm
06IH11c.HQ00010.P8SS	0.56	1.65	4.80	2.57	7.22	0.50	18.00	R1: 0.1/0.18...35µm
06IH11c.HQ00010.P8SS	0.50	1.54	4.08	2.32	7.88	2.01	18.00	R1: 0.1/0.18...35µm
06IH11c.HQ00010.P8SS	0.52	1.41	3.69	2.25	3.95	1.01	18.00	R1: 0.1/0.18...35µm
06IH11c.HQ00010.P8SS	0.50	1.35	3.28	2.06	3.67	3.02	18.00	R1: 0.1/0.18...35µm

Figure 7-10 Pressure and Feed rate velocity titration curves of [06IH11c.HQ00010.P8SS]

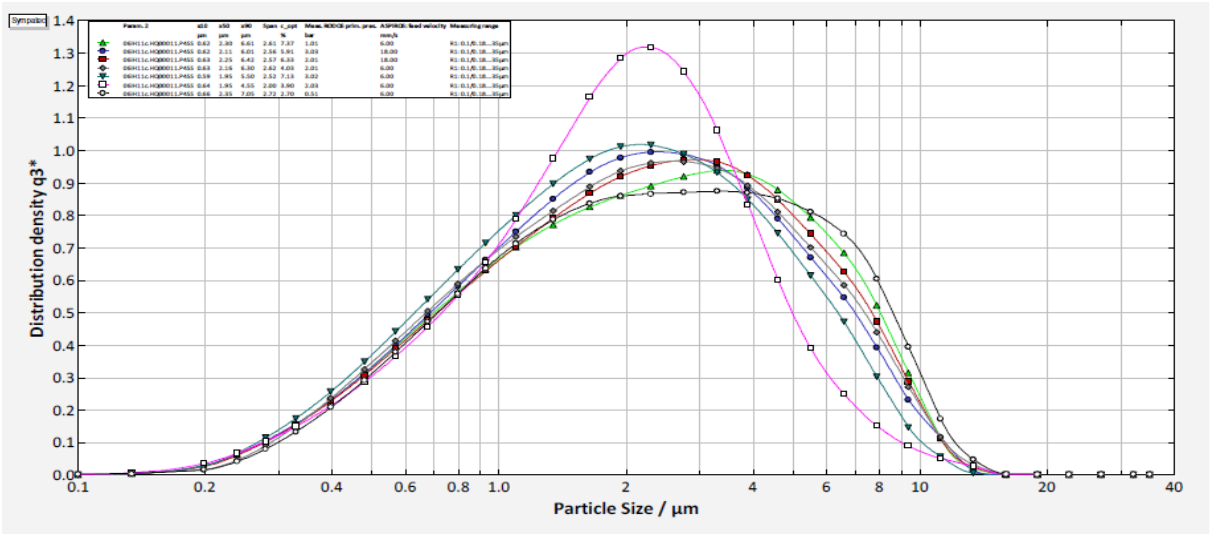
d) [IH11c] titration curves for [06IH11c.HQ00011.P3SS]



	Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
▲	06IH11c.HQ00011.P3SS	0.64	2.37	6.80	2.60	6.20	4.02	25.00	R1: 0.1/0.18...35µm
●	06IH11c.HQ00011.P3SS	0.57	1.78	5.15	2.56	7.43	6.01	25.00	R1: 0.1/0.18...35µm
■	06IH11c.HQ00011.P3SS	0.58	1.78	5.30	2.65	3.95	6.02	18.00	R1: 0.1/0.18...35µm
◆	06IH11c.HQ00011.P3SS	0.57	1.79	5.30	2.65	2.99	6.02	6.00	R1: 0.1/0.18...35µm

Figure 7-11 Pressure and Feed rate velocity titration curves of [06IH11c.HQ00011.P3SS]

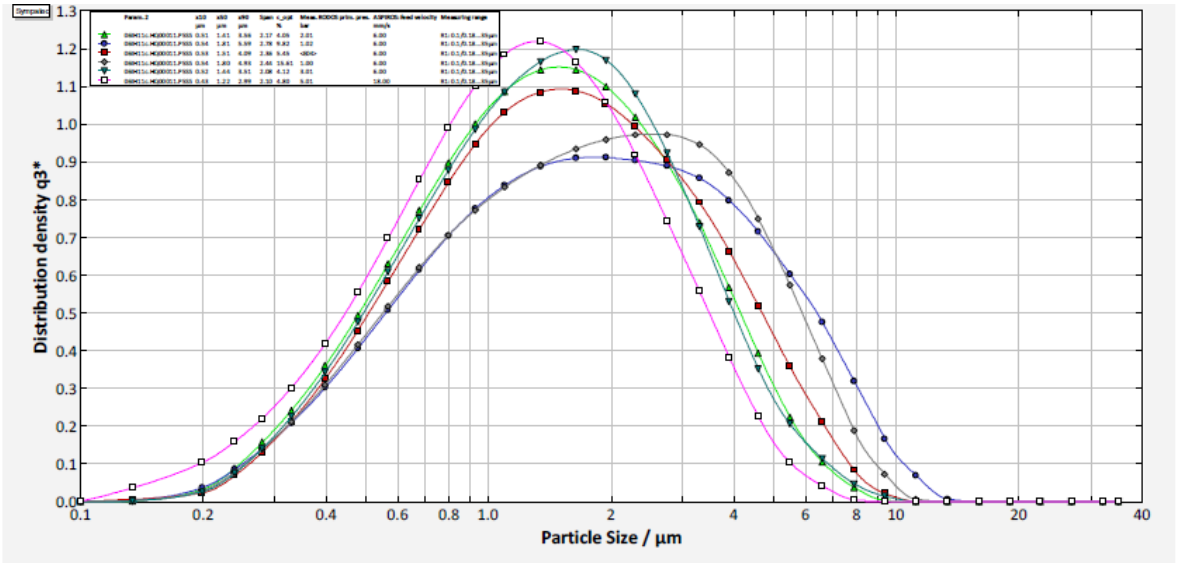
e) [IH11c] titration curves for [06IH11c.HQ00011.P4SS]



	Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
▲	06IH11c.HQ00011.P4SS	0.62	2.30	6.61	2.61	7.37	1.01	6.00	R1: 0.1/0.18...35µm
●	06IH11c.HQ00011.P4SS	0.62	2.11	6.01	2.56	5.91	3.03	18.00	R1: 0.1/0.18...35µm
■	06IH11c.HQ00011.P4SS	0.63	2.25	6.42	2.57	6.33	2.01	18.00	R1: 0.1/0.18...35µm
◆	06IH11c.HQ00011.P4SS	0.63	2.16	6.30	2.62	4.03	2.01	6.00	R1: 0.1/0.18...35µm
▼	06IH11c.HQ00011.P4SS	0.59	1.95	5.50	2.52	7.13	3.02	6.00	R1: 0.1/0.18...35µm
□	06IH11c.HQ00011.P4SS	0.64	1.95	4.55	2.00	3.90	2.03	6.00	R1: 0.1/0.18...35µm
○	06IH11c.HQ00011.P4SS	0.66	2.35	7.05	2.72	2.70	0.51	6.00	R1: 0.1/0.18...35µm

Figure 7-12 Pressure and Feed rate velocity titration curves of [06IH11c.HQ00011.P4SS]

f) [IH11c] titration curves for [06IH11c.HQ00011.P5SS]

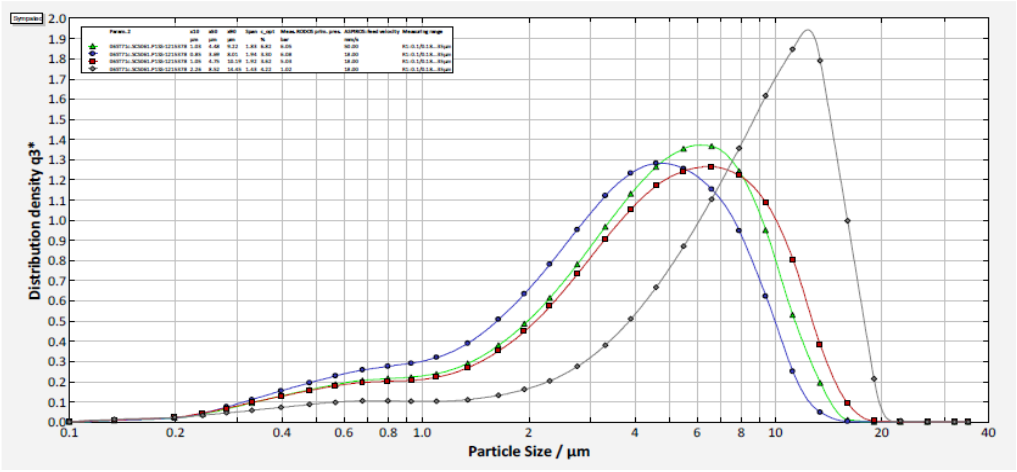


	Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
▲	06IH11c.HQ00011.P5SS	0.51	1.41	3.56	2.17	4.05	2.01	6.00	R1: 0.1/0.18...35µm
●	06IH11c.HQ00011.P5SS	0.54	1.81	5.59	2.78	9.82	1.02	6.00	R1: 0.1/0.18...35µm
■	06IH11c.HQ00011.P5SS	0.53	1.51	4.09	2.36	5.45	<E04>	6.00	R1: 0.1/0.18...35µm
◆	06IH11c.HQ00011.P5SS	0.54	1.80	4.93	2.44	15.61	1.00	6.00	R1: 0.1/0.18...35µm
▼	06IH11c.HQ00011.P5SS	0.52	1.44	3.51	2.08	4.12	3.01	6.00	R1: 0.1/0.18...35µm
□	06IH11c.HQ00011.P5SS	0.43	1.22	2.99	2.10	4.80	5.01	18.00	R1: 0.1/0.18...35µm

Figure 7-13 Pressure and Feed rate velocity titration curves of [06IH11c.HQ00011.P5SS]

IV. [ST71c] titration curves: Pressure and Feed rate velocity

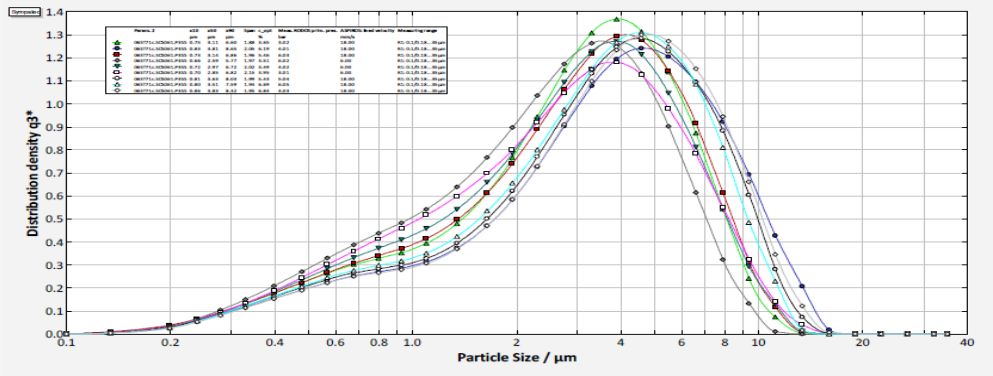
a) [ST71C] titration curves titration curves for [06ST71c.SCS061.P1SS]



Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
06ST71c.SCS061.P1SS-1215378	1.03	4.48	9.22	1.83	6.82	6.05	50.00	R1: 0.1/0.18...35µm
06ST71c.SCS061.P1SS-1215378	0.85	3.69	8.01	1.94	3.30	6.08	18.00	R1: 0.1/0.18...35µm
06ST71c.SCS061.P1SS-1215378	1.05	4.75	10.19	1.92	3.62	5.03	18.00	R1: 0.1/0.18...35µm
06ST71c.SCS061.P1SS-1215378	2.26	8.52	14.45	1.43	4.22	1.02	18.00	R1: 0.1/0.18...35µm

Figure 7-14 Pressure and Feed rate velocity titration curves of [06ST71c.SCS061.P1SS]

b) [ST71c] titration curves titration curves for [06ST71c.SCS061.P3SS]

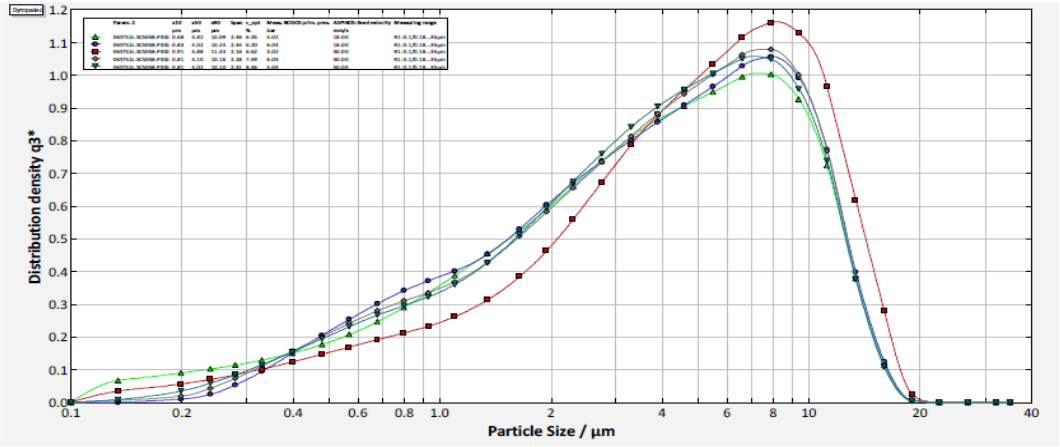


Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
06ST71c.SCS061.P3SS	0.75	3.11	6.60	1.88	3.65	3.02	18.00	R1: 0.1/0.18...35µm
06ST71c.SCS061.P3SS	0.83	3.81	8.65	2.05	6.19	4.01	18.00	R1: 0.1/0.18...35µm
06ST71c.SCS061.P3SS	0.73	3.14	6.86	1.96	5.46	6.03	18.00	R1: 0.1/0.18...35µm
06ST71c.SCS061.P3SS	0.66	2.59	5.77	1.97	3.51	6.02	6.00	R1: 0.1/0.18...35µm
06ST71c.SCS061.P3SS	0.72	2.97	6.72	2.02	3.49	4.02	6.00	R1: 0.1/0.18...35µm
06ST71c.SCS061.P3SS	0.70	2.85	6.82	2.15	3.95	3.01	6.00	R1: 0.1/0.18...35µm
06ST71c.SCS061.P3SS	0.81	3.63	8.03	1.99	5.43	5.04	18.00	R1: 0.1/0.18...35µm
06ST71c.SCS061.P3SS	0.80	3.51	7.59	1.94	6.69	6.05	18.00	R1: 0.1/0.18...35µm
06ST71c.SCS061.P3SS	0.86	3.83	8.32	1.95	6.84	4.03	18.00	R1: 0.1/0.18...35µm

Figure 7-15 Pressure and Feed rate velocity titration curves of [06ST71c.SCS061.P3SS]

V. [ST52c] titration curves: Pressure and Feed rate velocity

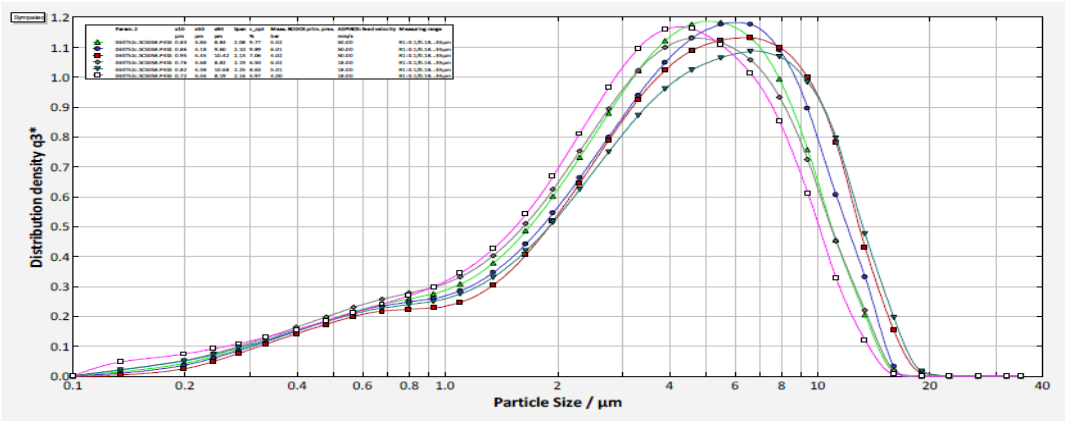
a) [ST52c] titration curves titration curves for [06ST52c.SCS058.P3SS]



Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
06ST52c.SCS058.P3SS	0.68	3.82	10.09	2.46	6.35	5.02	18.00	R1: 0.1/0.18...35µm
06ST52c.SCS058.P3SS	0.83	4.02	10.24	2.34	6.20	6.03	18.00	R1: 0.1/0.18...35µm
06ST52c.SCS058.P3SS	0.91	4.88	11.43	2.16	6.62	3.02	50.00	R1: 0.1/0.18...35µm
06ST52c.SCS058.P3SS	0.81	4.10	10.16	2.28	7.59	6.03	50.00	R1: 0.1/0.18...35µm
06ST52c.SCS058.P3SS	0.81	4.02	10.10	2.31	8.46	5.03	50.00	R1: 0.1/0.18...35µm

Figure 7-16 Pressure and Feed rate velocity titration curves of [06ST52c.SCS058.P3SS]

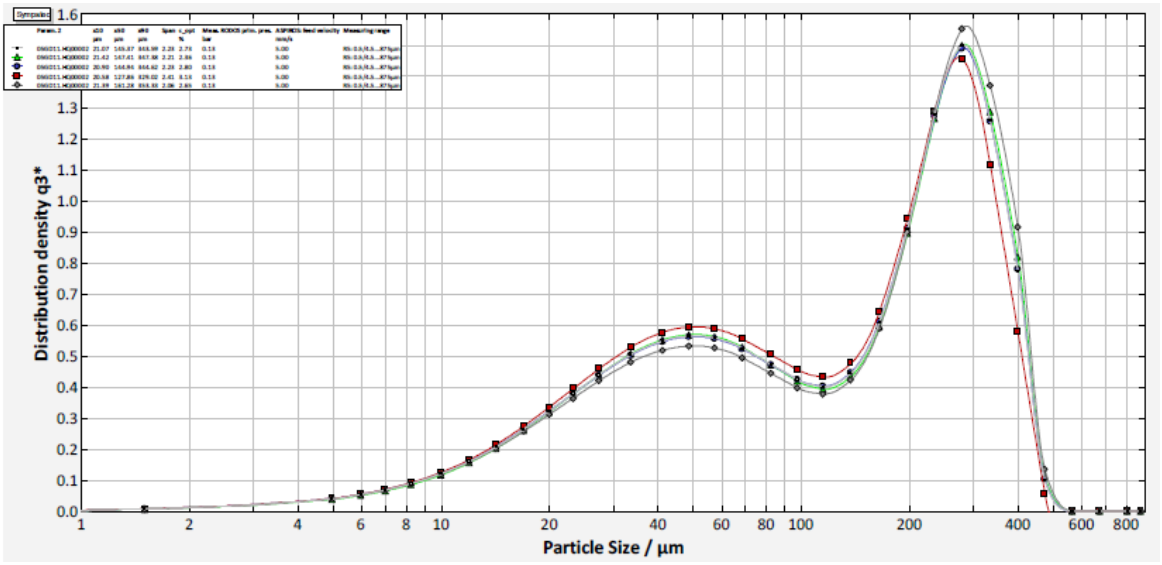
b) [ST52c] titration curves titration curves for [06ST52c.SCS058.P4SS]



Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
06ST52c.SCS058.P4SS	0.83	3.86	8.84	2.08	9.77	5.02	50.00	R1: 0.1/0.18...35µm
06ST52c.SCS058.P4SS	0.86	4.18	9.60	2.10	9.89	6.01	50.00	R1: 0.1/0.18...35µm
06ST52c.SCS058.P4SS	0.95	4.45	10.42	2.13	7.06	4.02	50.00	R1: 0.1/0.18...35µm
06ST52c.SCS058.P4SS	0.76	3.68	8.82	2.19	6.50	6.02	18.00	R1: 0.1/0.18...35µm
06ST52c.SCS058.P4SS	0.82	4.38	10.68	2.25	8.63	5.01	18.00	R1: 0.1/0.18...35µm
06ST52c.SCS058.P4SS	0.72	3.46	8.19	2.16	5.97	4.00	18.00	R1: 0.1/0.18...35µm

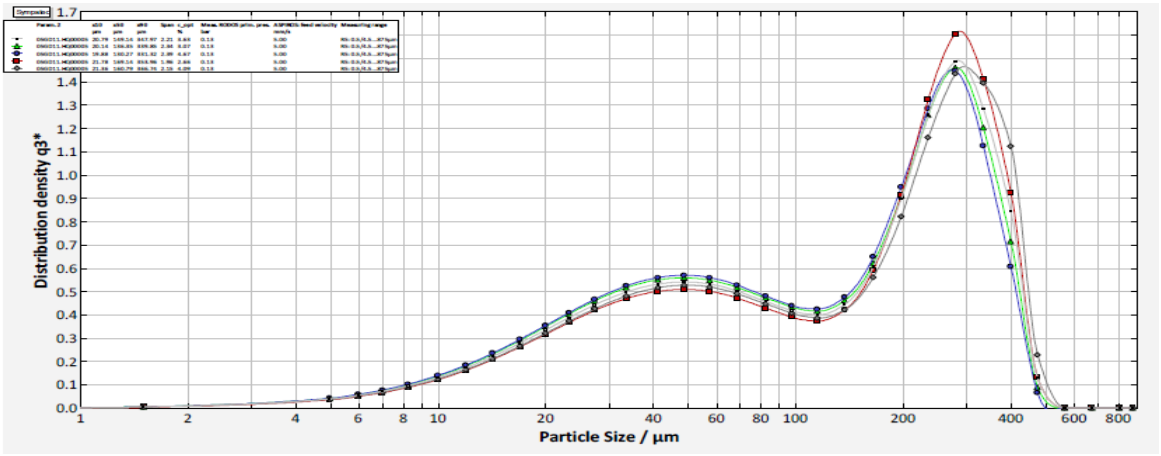
Figure 7-17 Pressure and Feed rate velocity titration curves of [06ST52c.SCS058.P4SS]

VI. [GD11] titration curves: Repeatability test



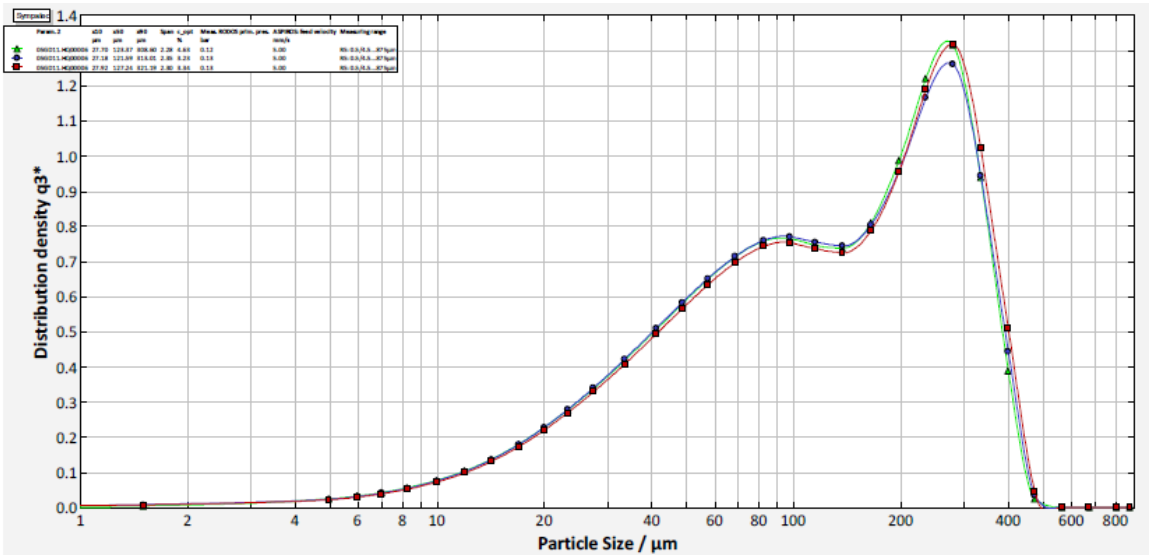
	Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
	05GD11.HQ00002	21.07	145.37	343.59	2.23	2.73	0.13	5.00	R5: 0.5/4.5...875µm
	05GD11.HQ00002	21.42	147.41	347.38	2.21	2.36	0.13	5.00	R5: 0.5/4.5...875µm
	05GD11.HQ00002	20.90	144.94	344.62	2.23	2.80	0.13	5.00	R5: 0.5/4.5...875µm
	05GD11.HQ00002	20.58	127.86	329.02	2.41	3.13	0.13	5.00	R5: 0.5/4.5...875µm
	05GD11.HQ00002	21.39	161.28	353.33	2.06	2.65	0.13	5.00	R5: 0.5/4.5...875µm

Figure 7-18 Repeatability test curves (0.1 bar; 5.00 mm/s) of [05GD11.HQ00002]



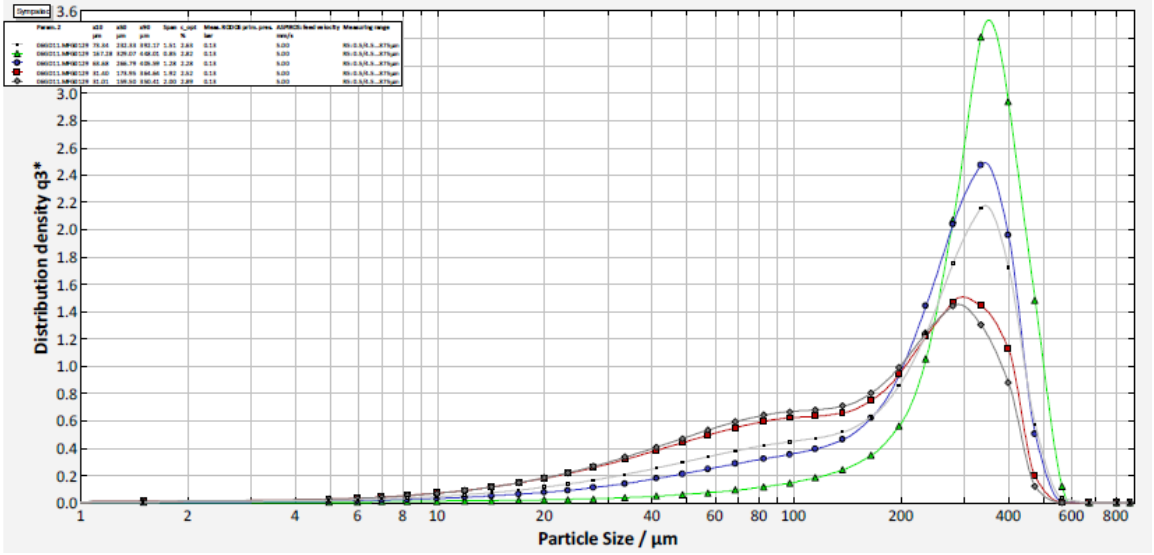
	Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
	05GD11.HQ00005	20.79	149.14	347.97	2.21	3.63	0.13	5.00	R5: 0.5/4.5...875µm
	05GD11.HQ00005	20.14	136.35	339.85	2.34	3.07	0.13	5.00	R5: 0.5/4.5...875µm
	05GD11.HQ00005	19.88	130.27	331.32	2.39	4.67	0.13	5.00	R5: 0.5/4.5...875µm
	05GD11.HQ00005	21.78	169.14	353.96	1.96	2.66	0.13	5.00	R5: 0.5/4.5...875µm
	05GD11.HQ00005	21.36	160.79	366.74	2.15	4.09	0.13	5.00	R5: 0.5/4.5...875µm

Figure 7-19 Repeatability test curves (0.1 bar; 5.00 mm/s) of [05GD11.HQ00005]



	Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
▲	05GD11.HQ00006	27.70	123.37	308.60	2.28	4.63	0.12	5.00	R5: 0.5/4.5...875µm
●	05GD11.HQ00006	27.18	121.59	313.01	2.35	3.23	0.13	5.00	R5: 0.5/4.5...875µm
■	05GD11.HQ00006	27.92	127.24	321.19	2.30	3.34	0.13	5.00	R5: 0.5/4.5...875µm

Figure 7-20 Repeatability test curves (0.1 bar; 5.00 mm/s) of [05GD11.HQ00006]



	Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
□	06GD11.MFG0129	73.34	232.33	392.17	1.51	2.63	0.13	5.00	R5: 0.5/4.5...875µm
▲	06GD11.MFG0129	167.28	329.07	448.01	0.85	2.82	0.13	5.00	R5: 0.5/4.5...875µm
●	06GD11.MFG0129	63.68	266.79	405.59	1.28	2.28	0.13	5.00	R5: 0.5/4.5...875µm
■	06GD11.MFG0129	31.40	173.95	364.64	1.92	2.52	0.13	5.00	R5: 0.5/4.5...875µm
◆	06GD11.MFG0129	31.01	159.50	350.41	2.00	2.89	0.13	5.00	R5: 0.5/4.5...875µm

Figure 7-21 Repeatability test curves (0.1 bar; 5.00 mm/s) of [06GD11.MFG0129]

VII. [GD11] solubility studies in water

The purposes of this study was to evaluate the particle morphology, size distribution and dissolution time of pilot scale (validation) and lab scale batches.

The equipment used in the solubility studies was Crystalline equipment (Figure 7-22). Crystalline is a specialized equipment for determining solubility curves and optimizing crystallization conditions in a fast way [36].

Figure 7-22 Crystalline Equipment (adapted from [36])

The first step was to determine the solubility curves of the production (GMP) and laboratory batches (non-GMP). Therefore, several tests were performed at different concentrations with sequences of heating and cooling ramps where the temperature ranged between 5º C and 50ºC.

After collecting the results, it was possible to determine the solubility curves, using an appropriate software: crystal-clear. In Figure 7-23 are presented the solubility curves for a laboratory batch (a [05GD11.HQ00006] and production batch (b) [06GD11.MFG0129].

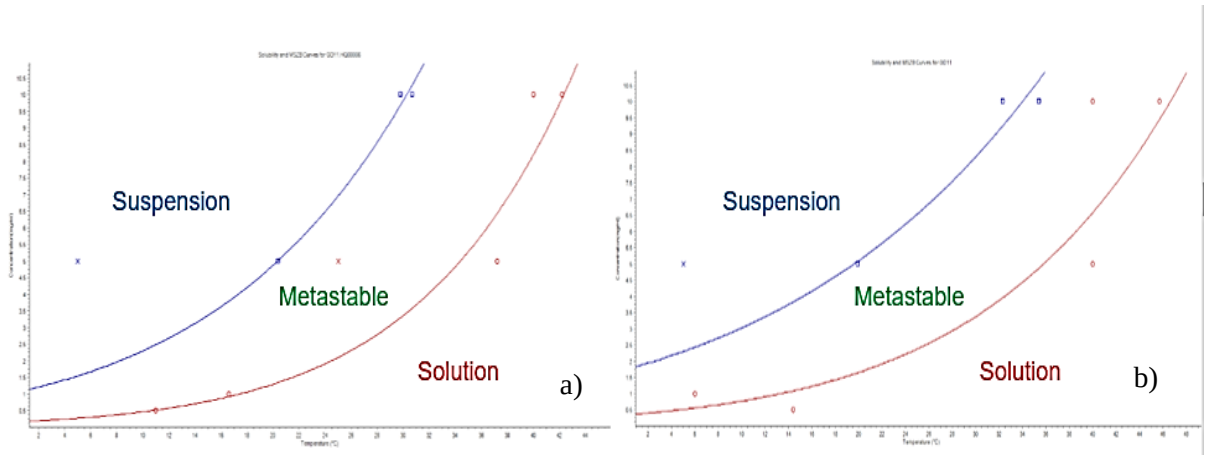


Figure 7-23 Solubility curves of a) [05GD11.HQ00006] and b) [06GD11.MFG0129]

As can be seen in Figure 7-23 the solubility curves are similar between production and laboratory batches.

The next step was to evaluate the dissolution time by creating a dissolution profile. Based on the solubility curves it was decided to choose a 5 mg/ml suspension of [GD11] in water at a dissolution temperature of 40ºC (heating ramp: 0.5ºC/min).

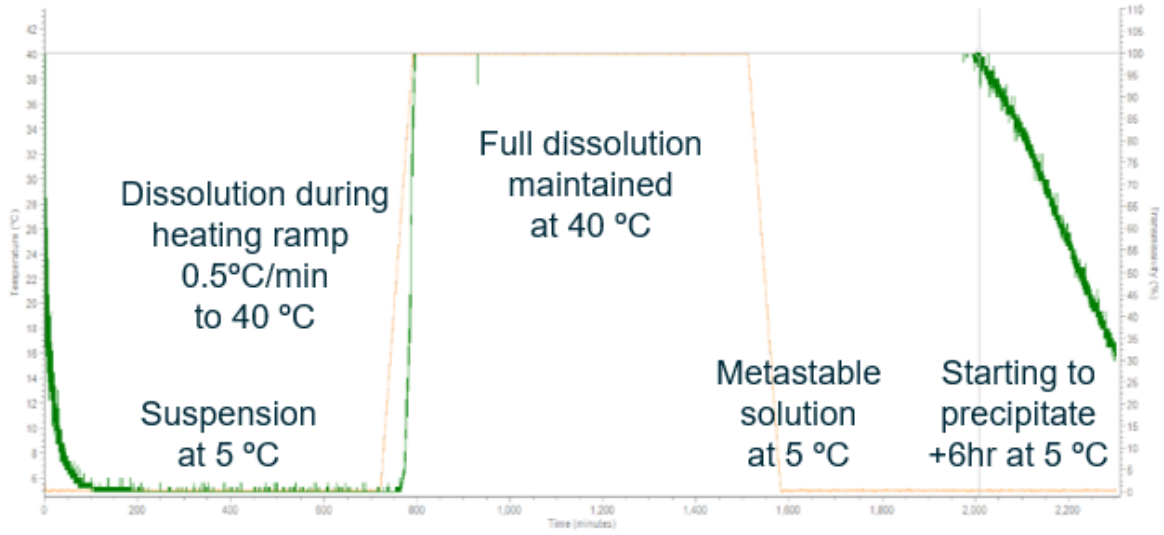


Figure 7-24 Temperature set for batch [05GD11.HQ00006] dissolution behavior analysis.

Figure 7-24 shows the temperature set (orange line) and transmissivity (green line) of the [05GD11.HQ00006] batch studied.

The transmissivity parameter relates to the percentage of the product that is dissolved in the solvent. A transmsiivity of 100% means a complete dissolution of the product while a transmissivity of 0% corresponds to a complete precipitation of the product.

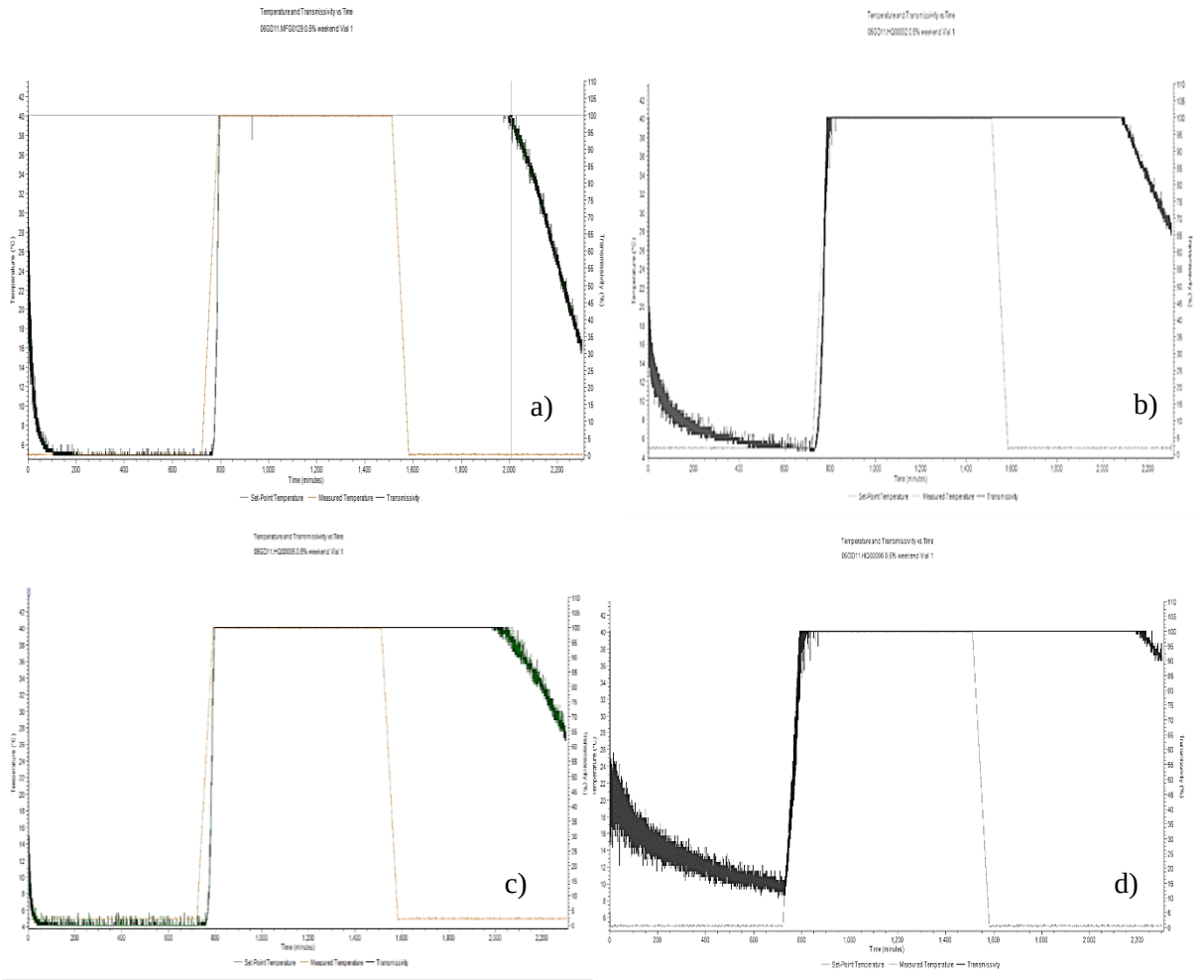


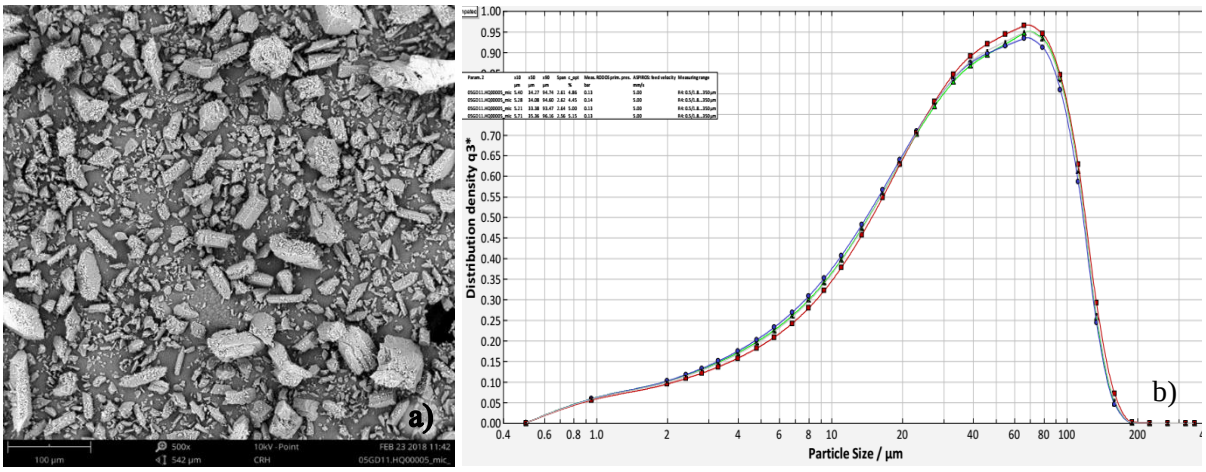
Figure 7-25 Dissolution time analysizs for batches a) [05GD11.HQ00002]; b) [05GD11.HQ00005]; c) [05GD11.HQ00006]; d) [06GD11.MFG0129].

The time to achieve a full dissolution was found to be similar between batches.

Table 7-7 Dissolution time for batches a) [05GD11.HQ00002]; b) [05GD11.HQ00005]; c) [05GD11.HQ00006]; d) [06GD11.MFG0129].

Batches	**Dissolution time (min)**
05GD11.HQ00002	96
05GD11.HQ00005	76
05GD11.HQ00006	72
06GD11.MFG0129	80
Mean Dissolution time (min)	81
RSD (%)	10

In order to assess the impact of a process alteration during the filtration step regarding the particle size and dissolution performance, batch [05GD11.HQ00005] was milled in a mortar. Smaller particles (worst case scenario) with an unimodal distribution were obtained and evaluated in SEM, for PSD (Sympatec) and dissolution time (Crystalline)



	Param. 2	x10 µm	x50 µm	x90 µm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
	05GD11.HQ00005_mic	5.40	34.27	94.74	2.61	4.86	0.13	5.00	R4: 0.5/1.8...350µm
	05GD11.HQ00005_mic	5.28	34.08	94.60	2.62	4.45	0.14	5.00	R4: 0.5/1.8...350µm
	05GD11.HQ00005_mic	5.21	33.38	93.47	2.64	5.00	0.13	5.00	R4: 0.5/1.8...350µm
	05GD11.HQ00005 mic	5.71	35.36	96.16	2.56	5.15	0.13	5.00	R4: 0.5/1.8...350µm

Figure 7-26 a) SEM analysis of micronized [05GD11.HQ00005]; b) Sympatec PSD analysis of micronized [05GD11.HQ00005].

The dissolution time evaluation of the micronized bacth was performed at the same concentration using the same temperature set.

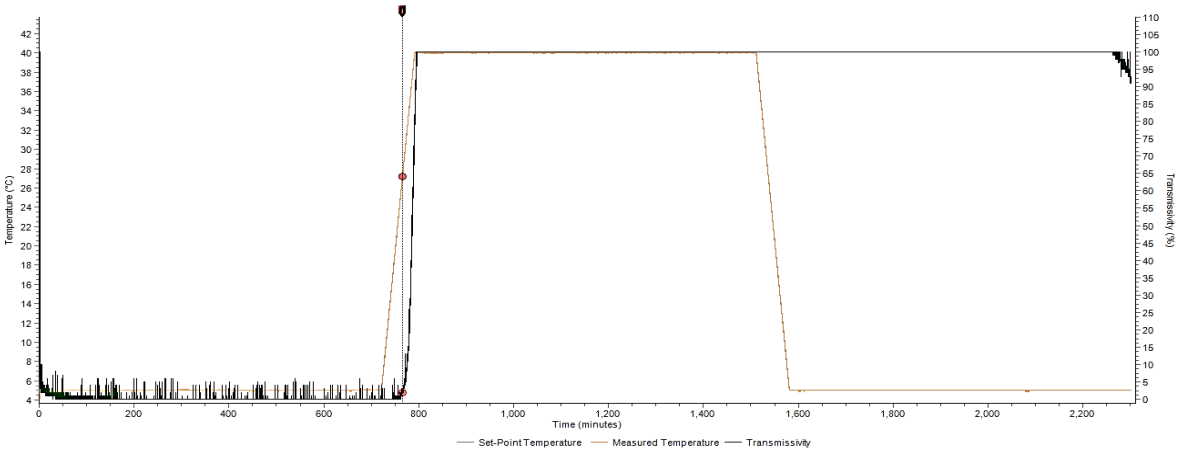


Figure 7-27 Dissolution time analysis of micronized [05GD11.HQ00005].

Table 7-8 Dissolution time of micronized [05GD11.HQ00005].

Batch	Dissolution time (min)
Mic.[05GD11.HQ00005]	46

As expected, the dissolution was faster than for unmilled product (46 min at 40ºC) for the same heating ramp (0.5ºC/min).